NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

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Description

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The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

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Processing of Starting samples

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2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

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1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

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The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

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- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

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4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

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3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

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NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

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2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
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4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

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Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

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NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

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For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

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1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

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Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

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2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

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For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

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- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

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1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

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2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

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4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

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3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

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NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

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2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
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4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

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All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

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NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

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1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

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Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

Numerous studies underscore the significant impact of blood sampling, handling, storage, and plasma preparation on both the yield and quality of DNA. Hence, maintaining consistency in the blood sampling procedure, handling, storage, and plasma preparation method is strongly advocated to ensure maximum reproducibility. The isolation of samples can be conducted following established protocols in the literature or by adhering to the recommendations given below:

For the preparation of **plasma from human EDTA blood**, follow these steps:

1. Subject a fresh blood sample to centrifugation for 10 minutes at 2,000 *x g*.
2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

- PCR signal output is boosted by incubating the elution fraction at higher temperatures. This is particularly significant if the template represents more than 20% of the total PCR reaction volume (e.g., over 4 µL of eluate used as a template in a PCR reaction with a total volume of 20 µL). With increased temperature incubation as described, the template can constitute up to 40% of the total PCR reaction volume.
- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

The specially designed funnel architecture of the NZY cfDNA Isolation columns enables elution volumes as minimal as 5-30 µL, yielding a highly concentrated DNA solution. The kit employs a cutting-edge bind-wash-elute methodology, commencing with a mixture of the sample and the binding buffer applied to the NZY cfDNA Isolation column. This allows the DNA to bind to a silica membrane. Two successive washing phases eliminate contaminants, ensuring the final elution comprises only the purest DNA, eluted with a buffer of 5 mM Tris-HCl, pH 8.5 (5–30 µL). The kit accommodates up to 240 µL of the sample in a single column loading phase, although DNA yield remains strongly dependent on the individual sample. With plasma, the yield typically varies from 0.1 ng to several hundred ng of DNA per mL sample. The kit can handle up to 720 µL of the sample with three column loadings, necessitating additional Lysis Buffer NCFB for samples exceeding 240 µL. Elution is achievable with as little as 5–30 µL of the elution buffer, rendering the DNA ready for downstream applications such as real-time PCR. The preparation time is approximately 15–30 minutes for 6–12 samples.

NZY cfDNA Isolation is recommended for forensic technologies. To ensure the prevention of DNA contamination, the kit is subject to a rigorously controlled production process and employs ethylene oxide (EO) treatment to eliminate any amplifiable DNA that might be introduced during the manufacturing process. This treatment ensures any DNA, potentially introduced during production, is inactivated, preventing accidental human profile generation via PCR amplification. Ethylene oxide treatment has proven to be the preferred method to avert DNA profile contamination.

Processing of Starting samples

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2. Carefully remove the plasma, ensuring no disturbance to the sedimented cells.
3. For storage prior to DNA isolation, freeze the plasma at -20 °C.
4. Before the DNA isolation process, thaw the frozen plasma samples and centrifuge them for 3 minutes at ≥ 11,000 *x g* to eliminate residual cells, cell debris, and particulate matter.
5. Use the supernatant for cfDNA isolation.

For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

Note: *The elution volume may be varied in a range of 5–30 µL. See section above for details on the correlation between elution volume, DNA concentration, and DNA amount eluted from the column.*

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Please note the following:

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- A 20 µL elution volume will evaporate down to 12–14 µL during an 8-minute heat incubation at 90 °C. If a higher final volume is required, please increase the initial volume of the elution buffer, for example, from 20 µL to 30 µL.
- Incubating the elution fraction at 90 °C for 8 minutes will denature DNA. If non-denatured DNA is needed (e.g., for downstream applications other than PCR like ligation or cloning), we recommend a longer incubation period at a temperature below 80 °C as most DNA has a melting point above 80 °C. For instance, you could incubate for 17 minutes at 75 °C.
- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

Considering the typically low DNA content, which results in a low overall quantity of isolated DNA, its fragmentation, and the absence of DNase inhibitors (note that the elution buffer does NOT contain EDTA), it is recommended to store the eluates on ice for short-term preservation and at -20 °C for long-term storage.

Storage conditions and reagents preparation

All components of the kit can be stored at 15–25 °C and will remain stable until the expiration date indicated on the package label. If any precipitation is noticed in the buffers, gently heat the buffer up to 25–37 °C to dissolve the precipitate before using it.

Prior to the kit's first usage, add the 1.35 mL of Proteinase Buffer to the lyophilized Proteinase K to dissolve it. The solution of Proteinase K, once prepared, can be stored at -20 °C for a minimum of 6 months.

Please be aware that Buffer NCFB includes guanidinium thiocyanate, which can generate highly reactive compounds when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NCFB	22 mL
Buffer NCFW	50 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin cfDNA Columns (red rings)	50
Collection tubes (2 mL)	100

High Sensitivity Protocol for cfDNA Isolation

Before starting the procedure, bring your sample to room temperature (15–25 °C) and ensure it is free of residual cells, cell debris, and particulate matter. This may require additional centrifugation of the plasma sample for 3 minutes at or above 11,000 *x g*. If you are following the high-sensitivity procedure, pre-set your thermal heating block to 75–90 °C for the final ethanol removal step (please refer to the above section for more details).

1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

   Adjust the Buffer NCFB volume if you use less than 240 µL of the sample (see below).

2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

3. **DNA binding condition adjustment:** Add 360 µL of Buffer NCFB (binding buffer). Remember to adjust the binding buffer volume based on your sample size, maintaining a 1:1.5 (v / v) ratio. If less than 240 µL sample is used, adjust the binding buffer volume accordingly.

4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.

NZY cfDNA Isolation kit

Catalogue numbers: MB46001, 50 columns

Description

The NZY cfDNA Isolation Kit, meticulously engineered for the extraction of circulating DNA from human blood plasma and other cell-free fluids, purifies DNA fragments ranging from 50 to 1000 base pairs (bp) with superior efficiency. A significant proportion of cell-free DNA in plasma originates from apoptotic cells, which often means a high degree of fragmentation. However, the fragmentation extent and the ratio of fragmented DNA to high molecular weight DNA are influenced by factors such as DNA origin (e.g., fetal, tumor, microbial DNA), the health of the blood donor, blood sampling procedure, and sample handling. Efficient isolation of the smallest DNA fragments is critical for the performance of many downstream applications. The NZY cfDNA Isolation purification system is optimized for this task, allowing the efficient isolation of highly fragmented DNA in the 50–1000 bp range. Designed with the capability of isolating fragmented cell-free DNA from human EDTA plasma, serum, and bronchial lavage, the NZY cfDNA Isolation Kit has proven successful with other cell-free fluids, including urine and follicular fluid.

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For the preparation of **other cell-free liquid samples (e.g., urine)**, adhere to the following procedure:

1. Clarify the sample using centrifugation (e.g., 5 minutes at 4,500 *x g*) to sediment cells or any other solid particles suspended in the sample.
2. Use only the supernatant for cfDNA isolation.

Elution procedures

The standard elution volume suggested is 20 µL. If you decrease the elution volume to between 5–15 µL, you will see an increase in DNA concentration, but this will be at the cost of total DNA yield. Conversely, extending the elution volume to 30 µL or more only slightly enhances total DNA yield, yet it reduces DNA concentration. A reduction in the standard 20 µL elution volume will heighten the concentration of residual ethanol in the eluate. For a 20 µL elution volume, it's advisable to heat incubate the elution fraction (i.e., incubate the eluate with the lid open for 8 minutes at 90 °C) when the eluate comprises more than 20% of the final PCR volume. This helps to prevent inhibition of sensitive downstream reactions.

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- If the initial volume of elution buffer applied to the column is less than 20 µL, reduce the time of heat incubation to avoid complete dryness.

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1. **Sample preparation:** Add 240 µL plasma or other cell-free fluid to a microcentrifuge tube (not included in the kit).

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2. **(Optional) Proteinase K treatment:** Mix 20 µL Proteinase K solution with the sample, then incubate at 37 °C for 10 minutes.

   Note: *This treatment may enhance PCR signal but could also affect the ratio of high to low molecular weight DNA.*

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4. **Sample mixing:** Invert the tube three times, vortex for 3 seconds, and briefly centrifuge to clean the lid.

5. **DNA binding:** Load the mixture (600 µL) into an NZYSpin cfDNA Column (red ring), placed in a 2 mL collection tube, and centrifuge.

   Note*: Maximum column volume is 600 µL. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution hasn't completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

6. **Membrane washing & drying:** Perform a first and second wash with 500 µL and 250 µL of Buffer NCFW, respectively. After each wash, centrifuge and discard the flow-through.

   Finally, place the column in a 1.5 mL microcentrifuge tube for elution (not included).

7. **DNA elution:** Add 20 µL of Buffer NE (Elution buffer) to the NZYSpin cfDNA Column (red ring) and centrifuge.

   Note: *Elution volume can range from 5-30 µL, depending on DNA concentration and the amount required (see section above for details).*

8. **Residual ethanol removal:** Heat the elution fraction with the lid open at 90 °C for 8 minutes to evaporate residual ethanol.

   Note: *Consider other incubation times and temperatures for specific residual ethanol removal needs (see section above for details).*

Rapid Protocol for cfDNA Isolation

The rapid procedure offers a balanced approach, efficiently optimizing DNA yield and concentration while also simplifying and accelerating the nucleic acid extraction process.

1. **Sample preparation:** Add 200 µL of plasma or alternative cell-free fluid to a microcentrifuge tube (not included). If less than 240 µL is available, adjust the binding buffer volume as per the guidelines.

2. **DNA binding condition adjustment:** Add 300 µL of Buffer NCFB (binding buffer). If less than 200 µL of sample is used, adjust the binding buffer volume, accordingly, ensuring a 1:1.5 (v/v) ratio between the sample and binding buffer.

3. **Sample mixing:** Invert the tube three times and vortex for 3 seconds. Briefly centrifuge the tube to remove any residue from the lid.

4. **DNA binding:** Load the 500 µL sample mixture onto a the NZYSpin cfDNA Column (red ring) situated in a 2 mL collection tube. Centrifuge at 11,000 *x g* for 30 seconds. Discard the flow-through in the collection tube and place the column into a new collection tube (provided).

   Note*: Maximum column volume is approximately 600 µL. Do not exceed to prevent spillage. If larger sample volumes need processing, repeat the loading step but note the increased risk of membrane clogging. If the solution has not completely passed the column, extend centrifugation for an additional 60 seconds at 11,000 x g.*

5. **Silica membrane washing and drying:** First Wash: Add 500 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*. Discard flow-through and place the column into a new collection tube (provided).

   Second Wash: Add 250 µL Buffer NCFW to the NZYSpin cfDNA Column (red ring). Centrifuge for 3 minutes at 11,000 *x g*. Discard flow-through and place the column into a 1.5 mL microcentrifuge tube for elution (not included).

6. **DNA elution:** Add 20 µL Buffer NE (elution buffer) to the NZYSpin cfDNA Column (red ring). Centrifuge for 30 seconds at 11,000 *x g*.