NZY FFPE gDNA Isolation Kit

Catalogue number	**Presentation**
MB49701	50 columns

Description

NZY FFPE gDNA Isolation Kit is designed for the efficient and rapid small-scale preparation of highly pure genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. This method utilizes a silica-based spin column and eliminates the need for flammable and aromatic substances such as xylene or d-limonene, which are typically used for deparaffinization. The procedure using in this kit avoids the challenging step of removing organic solvents from tissue pellets, which are often difficult to visualize. The kit employs optimized lysis buffers containing Proteinase K and SDS, enabling effective tissue digestion through an efficient two-phase process. In the first step, the paraffin in FFPE tissue sections is dissolved using the Buffer NPD. The tissue is then digested with a Proteinase K to solubilize the fixed material, releasing DNA into the solution. A subsequent heat incubation step with a specially formulated buffer efficiently removes crosslinks from the released DNA. Next, the ethanol is added to the lysate, which is loaded onto the NZYSpin HC FFPE column. After the DNA is absorbed into the silica membrane, two washing steps ensure the removal of salts, proteins, metabolites, and other cellular contaminants. Finally, pure genomic DNA is eluted, ready for downstream applications such as sequencing, PCR, multiplex-PCR, genotyping and other enzymatic manipulations.

Shipping and Storage conditions

The NZY FFPE gDNA Isolation Kit is shipped at room temperature. All kit components can be stored at room temperature (15-25 °C) and are stable till the expiry date if stored as specified.

Components

COMPONENT	MB49701 (50 COLUMNS)
Buffer NPD	25 mL
Buffer NPL	8 mL
Buffer ND	8 mL
Buffer NW5 (concentrate)	12 mL
Buffer NE	13 mL
Proteinase K (lyophilized)	30 mg
Proteinase Buffer	1.8 mL
NZYSpin HC FFPE Columns (green ring)	50
Collection tubes (2 mL)	100

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- 1,5 mL RNAse-free microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes
- Incubator at 60 °C
- Incubator at 90 °C

Specifications

Expected genomic DNA Yield: Strongly depends on sample quality and amount.

Sample material: The standard protocol allows the preparation of FFPE samples containing approximately 15 mg of paraffin. The recommended quantities of FFPE sections to use are presented in Table 1.

Table 1. Quantities, thickness, and area of FFPE samples.

Number of Sections	Thickness	Area
~17 sections	10 µm	100 mm²
~ 7 sections	10 µm	250 mm²
~ 5 sections	10 µm	325 mm²
~ 4 sections	10 µm	400 mm²
~ 3 sections	10 µm	575 mm²
~ 2 sections	10 µm	840 mm²
~ 1 section	10 µm	1680 mm²

Note: *For larger amounts of paraffin, please dissolve it by adding a higher volume of Buffer NPD to the starting sample (use 30 µL Buffer NPD per mg of paraffin).*

Columns type: silica membrane technology – high-concentration (HC) design

Elution Volume: 5-30 µL

Standard Protocol

Recommendations before starting

- Buffer NPL contains chaotropic salts. Wear gloves and goggles when using this kit.

Procedures before starting

- Proteinase K: add 1.35 mL of Proteinase Buffer to the Proteinase K vial and vortex. Proteinase K solution is stable at -20 °C for 6 months
- Buffer NW5: add 48 mL of 96-100% ethanol to the Buffer NW5 bottle.
- Preheat Buffer NE to 70 °C.

Protocol for DNA purification using Paraffin Dissolver Buffer

1. Sample preparation

Place FFPE section(s) into a 1,5 mL microcentrifuge tube.

Note: *See Table 1 to select the appropriate sample amounts.*

2. Deparaffinization of sample

Add 400 µL of Buffer NPD to the sample.

Incubate at 60 °C for 3 minutes. Vortex the sample immediately at a vigorous speed to dissolve the paraffin.

Note: *We recommend using a thermoshaker for incubation. Make sure that paraffin completely melts during the heat incubation step and mix well after melting to completely dissolve the paraffin. For samples containing more than 15 mg paraffin, refer to the note in the Specifications section. If more than 400 µL of Buffer NPD is necessary, place the sample in a 2 mL tube.*

Allow the sample to return to room temperature.

3. Lysis of sample

Add 100 µL Buffer NPL to the sample and mix by vortex vigorously. Centrifuge for 1 minute at 11,000 *x g*.

After centrifugation two phases will be visible: a lower (aqueous) phase and an upper (organic) phase. Tissue material will be transferred to the lower (aqueous) phase.

Note: *The upper (organic) phase can be removed and discarded after centrifugation.*

Pipette 10 µL Proteinase K solution directly into the lower (aqueous) phase. Mix the aqueous phase by pipetting up and down several times.

Notes:

- *Pipette up and down only the lower aqueous phase. Avoid mixing the lower phase and upper phase excessively.*
- *If multiple samples are processed, we recommend preparing a Buffer NPL/Proteinase K premix. Add 110 µL of the premix to the reaction tube, mix, and centrifuge to achieve the formation of two distinct phases. Pipette aqueous phase up and down several times to disperse the tissue in the lysis buffer.*
- *Make sure that the Proteinase K solution is mixed well with the lysis buffer NPL.*

Incubate at room temperature for 3 hours to lyse the sample.

Note: *If residual particles are visible after 3 hours, add additional 10 µL Proteinase K solution and continue digestion for further 3 hours or overnight.*

Vortex for five seconds.

Adjust the heating block to 90 °C.

Note: *At this point, the procedure can temporarily be stopped. If pausing, we recommend to store the samples at -20 °C.*

4. Decrosslink

Add 100 µL Buffer ND to the tube and vortex gently to mix Buffer ND into the aqueous (lower) phase.

Centrifuge for 30 seconds at 11,000 *x g* to obtain phase formation.

Incubate at 90 °C for exactly 30 minutes. Vortex 5 seconds and let cool down to room temperature (approx. 2 min).

Note: *This decrosslink step is necessary to remove the crosslinks (chemical modification caused by formalin) from the DNA, which was released into solution by the previous lysis step. Decrosslinked DNA generally shows better performance in downstream applications.*

5. Adjust binding conditions

Add 200 µL of 96-100% ethanol to the tube and mix immediately by vortex (2 x 5 seconds). Spin down briefly (~1 second at 1,000 *x g*) to achieve complete phase separation.

Note: *Avoid centrifuging at much higher g-force, because nucleic acid might precipitate.*

The ethanol will mix exclusively with the aqueous (lower) phase.

6. DNA Binding

Transfer the aqueous (lower) phase into the NZYSpin HC FFPE column.

Note: *It is recommended to pipette a volume of 450 µL on the column, to ensure that the complete aqueous (lower) phase is transferred (the volume of the aqueous phase is approx. 410 µL). Small carry-over of the organic (upper) phase has no negative effect on the binding procedure.*

Centrifuge for 30 seconds at 2,000 *x g* (if the solution does not flow through completely, centrifuge for 30 seconds at 11,000 *x g* until the complete solution passes the column).

Discard the flow-through and place the column in a new collection tube.

7. Wash and dry silica membrane

Add 400 µL of Buffer NW5 to the column. Centrifuge for 30 seconds at 11,000 *x g*. Discard the flow-through and place the column in a new collection tube.

Add 400 µL of Buffer NW5 to the column. Centrifuge for 2 minutes at 11,000 *x g* to dry the membrane. Discard the collection tube with flowthrough.

8. Elute DNA

Place the NZYSpin HC FFPE column into a clean microcentrifuge tube and add 20 µL of Buffer NE (range of elution volume: 5-30 µL) directly in the membrane column. Incubate 5 minutes at room temperature and centrifuge at >11,000 x *g* for 2 minutes to elute DNA.

Additionally, you can substitute Buffer NE (comprising 5 mM Tris/HCl, pH 8.5) with TE buffer or water. When using water, it is essential to verify and adjust the pH to fall within the range of 8 – 8.5. Deionized water commonly possesses a pH below 7, and it is worth noting that CO_2 absorption can lead to a decrease in the pH of unbuffered solutions. Hence, pH adjustment ensures the compatibility of the eluate with your downstream applications.

The genomic DNA can be stored at 4 °C or, preferably, at -20 °C.

9. <u>Optional</u>: Removal of residual ethanol

Incubate the eluate with open lid for 8 minutes at 90 °C.

Protocol for DNA purification using xylene reagent

1. Sample preparation

Place FFPE section(s) into a 1,5 mL microcentrifuge tube.

Note: *See Table 1 to select the appropriate sample amounts.*

2. Deparaffinization of sample

Add 1 mL of xylene (or an alternative reagent) to the sample.

Incubate at room temperature until the paraffin is completely dissolved (approx. 2 minutes) and vortex vigorously (10 seconds).

Note: *Make sure that paraffin is completely dissolved.*

Centrifuge at 11,000 x *g* for 2 minutes. Discard the supernatant by pipetting. Do not remove any of the pellet.

Add 1 mL 96-100% ethanol to the pellet and vortex.

Centrifuge at 11,000 x *g* for 2 minutes. Discard the supernatant by pipetting. Do not remove any of the pellet.

Incubate the open tube at 60 °C for 3-10 minutes to dry the pellet.

Note: *It is crucial to fully evaporate any residual ethanol, as its presence can reduce DNA yield.*

3. Lysis of sample

Add 100 µL Buffer NPL and 10 µL Proteinase K solution to the pellet and mix by vortex vigorously.

Note: *If multiple samples are processed, we recommend preparing a Buffer NPL/Proteinase K premix. Add 110 µL of the premix to the pellet.*

Centrifuge for 1 minute at 11,000 x *g*.

Note: *Solid section residuals ate the tube wall should be flushed back into the solution by pipetting. Pipette solution up and down in order to homogenize sections.*

Incubate at room temperature for 3 hours to lyse sample.

Note: *If residual particles are visible after 3 hours, add additional 10 µL Proteinase K solution and continue digestion for further 3 hours or overnight.*

Vortex for five seconds.

Adjust the heating block to 90 °C.

Note: *At this point, the procedure can temporarily be stopped. If pausing, we recommend to store the samples at -20 °C.*

4. Decrosslink

Add 100 µL Buffer ND to the lysate and vortex vigorously for 5 seconds.

Incubate at 90 °C for exactly 30 minutes.

Vortex 5 seconds and let cool down to room temperature (approx. 2 min).

Note: *This decrosslink step is necessary to remove the crosslinks (chemical modification caused by formalin) from the DNA, which was released into solution by the previous lysis step. Decrosslinked DNA generally shows better performance in downstream applications.*

5. Adjust binding conditions

Add 200 µL of 96-100% ethanol to the lysate and mix immediately by vortex (2 x 5 seconds).

Spin down briefly (~1 second at 1,000 x *g*) to clear the lid.

6. DNA Binding

Pipette the lysate up and down two times before transfer the lysate into the NZYSpin HC FFPE column.

Centrifuge for 30 seconds at 2,000 x *g* (if the solution does not flow through completely, centrifuge for 30 seconds at 11,000 x *g* until the complete solution passed the column).

Discard the flow-through and place the column in a new collection tube.

7. Wash and dry silica membrane

Add 400 µL of Buffer NW5 to the column.

Centrifuge for 30 seconds at 11,000 x *g*. Discard the flow-through and place the column in a new collection tube.

Add 400 µL of Buffer NW5 to the column.

Centrifuge for 2 minutes at 11,000 x *g* to dry the membrane. Discard the collection tube with flowthrough.

8. Elute DNA

Place the NZYSpin HC FFPE column into a clean microcentrifuge tube and add 20 µL of Buffer NE (range of elution volume: 5-30 µL) directly in the membrane column.

Incubate 5 minutes at room temperature and centrifuge at >11,000 x *g* for 2 minutes to elute DNA.

Additionally, you can substitute Buffer NE (comprising 5 mM Tris/HCl, pH 8.5) with TE buffer or water. When using water, it is essential to verify and adjust the pH to fall within the range of 8 – 8.5. Deionized water commonly possesses a pH below 7, and it is worth noting that CO_2 absorption can lead to a decrease in the pH of unbuffered solutions. Hence, pH adjustment ensures the compatibility of the eluate with your downstream applications.

The genomic DNA can be stored at 4 °C or, preferably, at -20 °C.

9. <u>Optional:</u> Removal of residual ethanol

Incubate the eluate with open lid for 8 minutes at 90 °C.

Quality control assay

All components of NZY FFPE gDNA Isolation Kit are tested following standard protocol.

Troubleshooting

LOW OR NO DNA YIELD

- **Sample Material**

Check if the sample was properly stored.

The quality of the sample significantly affects both the yield and purity of the DNA.

- **Inadequate Buffer preparation**

Check that Buffer NW5 concentrated was diluted with the correct volume of ethanol.

Ensure Proteinase K solution was properly prepared.

- **Proteinase K digestion time**

Optimal digestion time, ranging from 3 to 16 hours, depends on the sample type and must be determined experimentally. If tissue remains undigested after 3 hours, extend incubation up to 16 hours and consider adding more Proteinase K after the initial 3-hour period.

CLOGGED COLUMNS

- **Large amount of sample material**

Check if the amount of starting material used is recommended. Do not use a large amount of sample.

- **Insufficient Homogenization**

Ensure thorough homogenization of the sample material.

DEGRADED DNA

- **DNase Contamination**

Starting sample was not stored properly.

Check your working area and pipettes for possible DNase contamination. Implement stringent cleanliness protocols.

SUBOPTIMAL PERFORMANCE IN DOWNSTREAM APPLICATIONS

- **Carry-over of ethanol or salt**

Ensure the flowthrough does not touch the column after the second ethanol wash.
Check if Buffer NW5 was properly prepared and used at room temperature, as lower temperatures during the washing step can reduce the efficiency of salt removal by Buffer NW5.