NZY Tissue gDNA Isolation kit

Catalogue numbers:

MB13502, 50 columns
MB13503, 200 columns

Description

NZY Tissue gDNA Isolation kits are designed for the simple and rapid small-scale preparation of highly pure genomic DNA from a variety of sample sources including animal cells and tissues, Gram-positive and Gram-negative bacteria, mouse tails, yeast, forensic samples and clinical samples (e.g., whole blood, serum, plasma or body fluids). The method is spin column silica-based and requires no phenol or chloroform extraction. This kit uses optimized lysis buffers containing Proteinase K and SDS to release DNA from cells. After preparing the lysate, DNA is selectively absorbed into the NZYSpin Tissue Column and others impurities such as proteins and salts are removed during the washing steps. The eluted genomic DNA has a $A_{260/280}$ ratio between 1.7 and 1.9 what makes it ready to use in applications like sequencing, PCR, multiplex-PCR, genotyping and a wide range of other enzymatic manipulations.

The NZY Tissue gDNA Isolation kit is optimized to isolate up to 35 µg of DNA from up to 25 mg of tissue samples or 10^7 cells. We suggest not using more than the recommended starting material to prevent reduction in yield and purity of DNA isolated. For samples with very high RNA and protein contents (e.g., liver or spleen tissues), use only up to 15 mg of the sample. This kit is suitable for isolation of DNA from human or animal blood.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date. Before use, add 1.35 mL (MB13502/3) of Proteinase buffer to each vial of Proteinase K and vortex. Proteinase K solution is stable at -20 °C for up to 6 months. Add 28 mL (MB13502) or 100 mL (MB13503) of ethanol (96-100%) to each bottle of buffer NW2. Buffers NL and NW1 contain guanidine hydrochloride. Wear gloves and goggles when using this kit.

System Components

Component	50 columns	200 columns
Buffer NT1	20 mL	80 mL
Buffer NL	15 mL	60 mL
Buffer NW1	30 mL	120 mL
Buffer NW2 (concentrate)	2 x 7 mL	2 x 25 mL
Buffer NE	15 mL	60 mL
Proteinase K (lyophilized)	30 mg	4 x 30 mg
Proteinase buffer	1.8 mL	7 mL
NZYSpin Tissue columns (light green ring)	50	200
Collection tubes (2 mL)	100	400

Standard protocol for isolating genomic DNA from animal tissues, cultured cells and bacteria cells

1. Sample preparation

Animal Tissues: Cut up to 25 mg tissue sample into small pieces, and place it in a microcentrifuge tube. Proceed with step 2.

Notes: *Tissue samples can be ground under liquid nitrogen for more efficient lysis.*
For rodent tails, place one (for rat) or two (for mouse) 0.6 cm-long pieces in a 1.5 mL tube.

Cultured Cells: Re-suspend up to 10^7 cells in 200 µL Buffer NT1. Add 25 µL Proteinase K solution and 200 µL Buffer NL*. Mix thoroughly by vortex, and incubate at 56 °C for 10-15 min. Vortex occasionally during incubation. Proceed with step 5.

**Mix Buffer NL thoroughly by shaking before use.*

Bacteria Cells: Pellet up to 1 mL bacteria culture for 5 min at 8,000 x*g*. Discard supernatant. Re-suspend cell pellet in 180 µL Buffer NT1 by pipetting up and down. Add 25 µL Proteinase K solution and vortex vigorously. Incubate at 56 °C for 1-3 hours. Mix occasionally during incubation. Proceed with step 3.

Clinical samples: Use up to 200 µL whole blood, plasma, serum, buffy coat or body fluids. Add 25 µL of Proteinase K solution to a 200 µL clinical sample in a microcentrifuge tube. Add 200 µL Buffer NL* to the sample and mix vigorously by vortex. Incubate at 56 °C for 10-15 min. Proceed with step 5.

Notes: *For leukocyte rich samples like buffy coat, use smaller volumes and dilute the samples with sterile PBS buffer.*

**Mix Buffer NL thoroughly by shaking before use.*

For other sample sources see the support procedures available in https://www.nzytech.com/products-services/kits-genomic-dna-purification/mb135/.

2. Pre-lysis of sample

Add 180 µL of Buffer NT1 and 25 µL Proteinase K solution to the sample. Mix thoroughly by vortex. Incubate at 56 °C for 1-3 hours and vortex occasionally during incubation.

Note: *Samples that are difficult to lyse can be incubated overnight as well.*

3. Removal of RNA (optional)

If RNA-free DNA is required, add 10 µL of RNase A (40 mg/mL) solution (not included, available as MB18701 – NZY RNAse A, 100 mg) to each sample. Mix and incubate for 5 min at room temperature.

4. Lysis of sample

Vortex the sample. Add 200 µL Buffer NL* to the sample, and mix by vortex for 10 seconds.

Notes: *If insoluble particles are visible, centrifuge for 5 min at full speed and transfer the supernatant to a new microcentrifuge tube. *Mix Buffer NL thoroughly by shaking before use.*

5. Addition of ethanol

Add 210 µL of 100% ethanol to the sample and mix immediately by vortex.

6. DNA binding

Transfer the mixture from step 5 into a NZYSpin Tissue Column placed in a 2 mL collection tube. Centrifuge for 1 min at > 11,000 x*g*. Discard flow-through and place the column in a new collection tube.

7. Wash silica membrane

Add 500 µL of Buffer NW1 to the column. Centrifuge for 1 min at > 11,000 x*g*. Discard flow-through and place the column back into the collection tube.

Add 600 µL of Buffer NW2 (make sure ethanol was previously added) to the column, and centrifuge for 1 min at > 11,000 x*g*. Discard flow-through.

Note: *For isolations of viral DNA from stool, we recommend to repeat the wash silica membrane step with Buffer NW2*. Add more 600 µL of Buffer NW2 to the column, and centrifuge for 1 min at > 11,000 x*g*. Discard flow-through.

8. Dry silica membrane

Place the NZYSpin Tissue Column back into the collection tube and centrifuge for 2 min at > 11,000 x*g*.

9. Elute DNA

Place the column into a clean microcentrifuge tube and add 100 µL of Buffer NE, TE buffer or sterile water (preheating of elution buffer to 70 °C may improve yield) directly in the membrane column. Incubate 1 min at room temperature and centrifuge at >11,000 x*g* for 2 min to elute DNA. The genomic DNA can be stored at 4 °C or -20 °C.

Data:

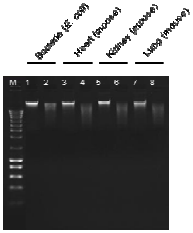

Fig. 1. Genomic DNA from various sources isolated with NZY Tissue gDNA Isolation kit. 0.25 µg of each isolated DNA was analysed on a 0.8% agarose gel. Lanes 2, 4, 6 and 8: gDNA digested with *Eco*R I. Lanes 1, 3, 5 and 7: undigested gDNA. M: NZYDNA Ladder III (MB0440); 1, 2: gDNA from bacteria (*E. coli*); 3, 4: gDNA from mouse heart; 5, 6: gDNA from mouse kidney; 7, 8: gDNA from mouse lung.

Quality control assay

Functional assay

All components of NZY Tissue gDNA Isolation kit are tested following the isolation protocol described above. The purification system must isolate 25-35 µg of gDNA/column.

V2101

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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