MB135_IFU_EN_V2401

NZY Tissue gDNA Isolation kit

Catalogue number	Presentation
MB13502	50 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 A260/280 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.

Shipping & Storage Conditions

This product 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	MB13502 (50 COLUMNS)
Buffer NT1	20 mL
Buffer NL	15 mL
Buffer NW1	30 mL
Buffer NW2 (concentrate)	2 x 7 mL
Buffer NE	15 mL
Proteinase K (lyophilized)	30 mg
Proteinase buffer	1.8 mL
NZYSpin Tissue columns (light green ring)	50
Collection tubes (2 mL)	100

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- 1,5 mL microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes
- Vortex
- Homogenization/disruptor system

Specifications

Expected genomic DNA Yield: This protocol was designed for purification up to 35 µg of pure DNA (from up to 25 mg of tissues or up to10^7 cells) with an A_{260}/A_{280} ratio between 1.7 and 1.9.

Columns type: silica membrane technology

Elution Volume: 40-100 µL

Standard Protocol

Recommendations before starting

- Buffers NL and NW1 contain guanidine hydrochloride. Wear gloves and goggles when using this kit.

Procedures before starting

Reagents Preparation

- 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 up to 6 months.
- Buffer NW2: add 28 mL of 96-100% ethanol to the Buffer NW2 bottle.
- Preheat Elution Buffer NE to 70 °C.

Procedure

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.*
- *For lipid-rich samples, such as brain tissues, the extraction of genomic DNA can be difficult. Please centrifuge the crude lysate to separate the lipids from the aqueous phase. Pierce the fatty layer with the pipet tip to aspirate the cleared liquid. Proceed with step 2.*

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 Buffer NL thoroughly by shaking before use). Mix thoroughly by vortex and incubate at 56 °C for 10-15 min. Vortex occasionally during incubation. Proceed with step 5.

Bacteria Cells: Pellet up to 1 mL bacteria culture for 5 min at 8,000 xg. Discard supernatant. Re-suspend cell pellet in 180 µL Buffer NT1 by pipetting up and down.

Note: *For bacteria that are more difficult to lyse, such as Gram-positive bacteria, a preincubation step with a lytic enzyme (e.g. lysozyme) is needed. Resuspend the pellet cells in the following buffer, instead of Buffer NT1: 20 mM Tris/HCl, 2 mM EDTA, 1% Triton X-100, pH 8.0, supplemented with lysozyme at 20 mg/mL, and incubate for 30-60 min at 37 °C.*

Add 25 µL Proteinase K, incubate at 56 °C until complete lysis. 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 (mix Buffer NL thoroughly by shaking before use) to the sample and mix vigorously by vortex. Incubate at 56 °C for 10-15 min. Proceed with step 5.

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

Depending on the starting material, sample preparation may require adaptation/optimization to increase the efficiency of this important initial step. For some types of starting material samples, we offer a support protocol available at 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 (mix Buffer NL thoroughly by shaking before use) to the sample and mix by vortex for 10 seconds.

Note: *If insoluble particles are visible, centrifuge for 5 min at full speed and transfer the supernatant to a new microcentrifuge tube.*

5. Addition of ethanol

Add 210 µL of 96-100% 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 xg. 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 DNA from stool, we recommend repeating 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 xg. 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 NZYSpin Tissue column into a clean microcentrifuge tube and add 100 µL of Buffer NE (preheated to 70 °C) directly in the membrane column. Incubate 5 min at room temperature and centrifuge at >11,000 x*g* for 2 min to elute DNA. You have the flexibility to tailor the elution method and the elution buffer volume to suit your specific application needs:

- **Complete Yields:** To achieve comprehensive yields, perform two elution steps using 2 × 100 µL each, which allows for the retrieval of approximately 90 – 100% of the bound nucleic acids. Afterward, combine the eluates and measure the total yield.
- **Highly Concentrated Eluates:** If your application requires highly concentrated eluates, opt for minimal elution volumes ranging from 40 to 60 µL. This approach typically yields around 60 – 80% of the bound nucleic acids, producing highly concentrated eluates.

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.

Quality control 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, depending on the source of the tested samples.

Troubleshooting

LOW OR NO DNA YIELD
• **Insufficient Homogenization/Incomplete lysis**
Ensure thorough homogenization of the sample material. Make sure that the sample was mixed with Buffer NT1/Proteinase K in accordance with above recommendations.
• **Inadequate Buffers preparation**
Check that Buffer NW2 concentrated was diluted with correct volume of ethanol.
• **Excessive RNA**
If the sample contains an excessive amount of RNA, add 10 – 20 µL of RNase A solution to the lysis buffer following heat incubation. If needed, consider adding the enzyme to the cleared lysate and incubate for 30 minutes at 37 °C.
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.

SUBOPTIMAL ELUTION
• **Low Elution Volume**
To improve elution efficiency, consider increasing the elution volume, with options of up to 200 µL. Alternatively, repeat the elution step up to three times. Ensure that the elution buffer is preheated to 70 °C before use.
• **pH of Elution Buffer**
Verify the pH of the elution buffer, which should fall within the range of pH 8.0 – 8.5. To guarantee the correct pH, use the provided Elution Buffer NE (5 mM Tris / HCl, pH 8.5).
DEGRADED DNA
• **DNase Contamination**
Starting sample was not stored properly. Follow recommendations for storage and handling of your sample type. Check your working area and pipettes for possible DNase contamination. Implement stringent cleanliness protocols.
• **Excessive Centrifugation Speed**
Ensure that you centrifuge at the speed specified in the protocol. Higher velocities and prolonged vortexing can result in DNA shearing.
LOW DNA QUALITY
• **Contaminants in Sample**
If the sample contains DNA-degrading contaminants such as phenolic compounds or metabolites, consider repeating the washing step with Buffer NW1 to improve DNA quality.
• **Presence of RNA**
If the sample contains an excessive amount of RNA, add 10 – 20 µL of RNase A solution to the lysis buffer following heat incubation. If needed, consider adding the enzyme to the cleared lysate and incubate for 30 minutes at 37 °C.
• **Low A_{260}/A_{280} ratio**
Check that Buffer NW2 concentrated was prepared correctly. Ensure that Buffers NW1 and NW2 are used correctly. If the A_{260}/A_{280} ratio of the DNA eluted is below than 1.6, repeat the purification protocol.
• **Low A_{260}/A_{230} ratio**
Probably the eluate contains carry-over of ethanol or salt. Ensure that centrifugation steps were done at ≥ 1 min at 11,000 *xg* to remove all traces of wash buffers. If necessary, repeat the centrifugation step.

By addressing these possible causes and implementing the suggested solutions, you can troubleshoot common issues encountered during the DNA isolation process and achieve better results with the NZY Tissue gDNA Isolation kit.