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NZYtech genes & enzymes

Instructions for use

MB517 _IFU_EN_V2501

NZY Tissue, Blood & Cells gDNA Isolation Kit

Catalogue number	Presentation
MB51701	50 columns
MB51702	4 x 50 columns

FEATURES

- Supreme Proteinase K–enhanced lysis provides rapid and efficient DNA release from even challenging sample types.
- Streamlined silica-spin workflow delivers fast, robust purification with minimal hands-on time.
- Optimized chemistry ensures consistently high DNA yields and excellent purity for demanding downstream applications.
- Broad versatility as a single kit for tissues, cultured cells, bacteria, and clinical samples with highly reproducible results.

Description

NZY Tissue, Blood & Cells gDNA Isolation Kit is designed for the rapid and efficient purification of high-quality 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 based on the usage of silica-based spin column and requires no phenol or chloroform extraction, thereby ensuring a safe and efficient protocol. This kit uses an optimized lysis buffer containing Supreme Proteinase K to release DNA from cells. Extensive Research and Development was devoted to fine-tuning Supreme Proteinase K and the lysis chemistry, resulting in markedly faster digestion and consistently higher DNA recovery, even from challenging samples. This optimized formulation shortens incubation times without compromising DNA integrity, enabling laboratories to accelerate their workflows. By combining speed with robust yields, the kit offers a reliable solution for routine and demanding genomic applications alike. After preparing the lysate, DNA is selectively bonded to the NZYSpin DNA Column and proteins and salts impurities are efficiently removed during the washing steps. The eluted genomic DNA presents an A260/280 ratio between 1.7 and 1.9 making it ready to use in applications such as sequencing, PCR, multiplex-PCR, genotyping, and a wide range of other enzymatic manipulations.

NZY Tissue, Blood & Cells gDNA Isolation Kit is optimized to isolate up to 30 µg of DNA from up to 25 mg of tissue samples or 1 x 10^7 cells. It is not advisable to use 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 the 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	MB51701 (50 COLUMNS)
Buffer NT2	20 mL
Buffer NL2	15 mL
Buffer NW3	30 mL
Buffer NW4	12 mL
Buffer NE2	15 mL
Supreme Proteinase K solution	1.5 mL
NZYSpin DNA columns	50
Collection tubes	100

Materials and Instrumentation 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 the purification of up to 30 µg of pure DNA (from up to 25 mg of tissues or up to 1 x 10^7 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 NL2 and NW3 contain guanidine hydrochloride. Wear gloves and goggles when handling this kit.

Procedures before starting

Reagents Preparation

- Buffer NW4: add 48 mL of 96-100% ethanol to the Buffer NW4 bottle.
- Preheat Elution Buffer NE2 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. 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: Resuspend up to 10^7 cells in 180 µL Buffer NT2. Add 25 µL Supreme Proteinase K solution and 200 µL Buffer NL2 (mix Buffer NL2 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 bacterial culture for 5 min at 8,000 *xg*. Discard the supernatant. Re-suspend the cell pellet in 180 µL Buffer NT2 by pipetting up and down. Add 25 µL Supreme Proteinase K solution, incubate at 56 °C for 30 min. Mix occasionally during incubation. **Proceed with step 3**.

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 cell pellet in the following buffer, instead of Buffer NT2: 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 Supreme Proteinase K solution, 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 Supreme Proteinase K solution to a 200 µL clinical sample in a microcentrifuge tube. Add 200 µL Buffer NL2 (mix Buffer NL2 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.

2. Pre-lysis of sample

Add 180 µL of Buffer NT2 and 25 µL Supreme Proteinase K solution to the sample. Mix thoroughly by vortex. Incubate at 56 °C for 30 minutes and vortex occasionally during incubation.

Note: *Samples that are difficult to lyse can be incubated for a longer time, up to overnight.*

3. RNA Removal (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) to each sample. Mix and incubate for 5 min at room temperature.

4. Lysis of sample

Vortex the sample. Add 200 µL Buffer NL2 (mix Buffer NL2 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 DNA Column placed in a 2 mL collection tube. Centrifuge for 1 min at > 13,000 *xg.* Discard flow- through and place the column in a new collection tube.

7. Wash silica membrane

Add 500 µL of Buffer NW3 to the column. Centrifuge for 1 min at > 13,000 x*g*. Discard the flow-through and place the column back into the collection tube.

Note: *For blood samples, we recommend repeating the silica membrane wash step with Buffer NW3. Add an additional 500 µL of Buffer NW3 to the column, and centrifuge for 1 min at > 13,000 xg. Discard the flow-through.*

Add 600 µL of Buffer NW4 (make sure ethanol was previously added) to the column, and centrifuge for 1 min at > 13,000 x*g*. Discard the flow- through.

Note: *For isolation of DNA from stool or blood samples, we recommend repeating the silica membrane wash step with Buffer NW4. Add an additional 600 µL of Buffer NW4 to the column, and centrifuge for 1 min at > 13,000 xg. Discard the flow-through.*

8. Dry silica membrane

Place the NZYSpin DNA Column back into the collection tube and centrifuge for 2 min at high-speed (> 13,000 xg).

9. Elute DNA

Place the NZYSpin DNA column into a clean microcentrifuge tube and add 100 µL of Buffer NE2 (preheated to 70 °C) directly onto the silica membrane. Incubate for 5 min at room temperature and centrifuge at >13,000 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 NE2 (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.

Data

The data presented in this section summarize the performance of the NZY Tissue, Blood & Cells gDNA Isolation Kit, in extraction of genomic DNA from multiple matrices.

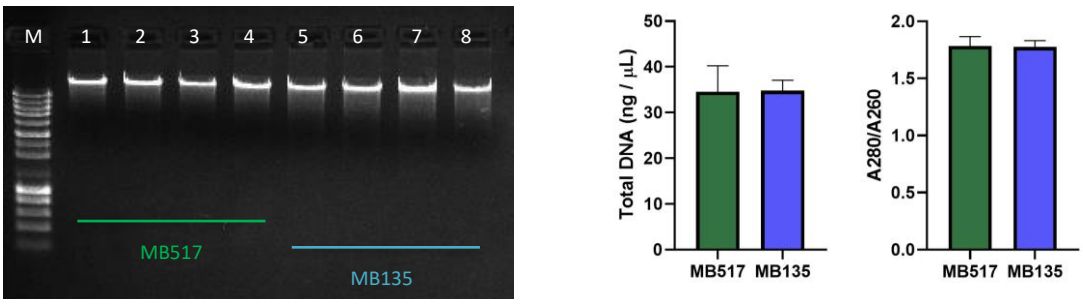
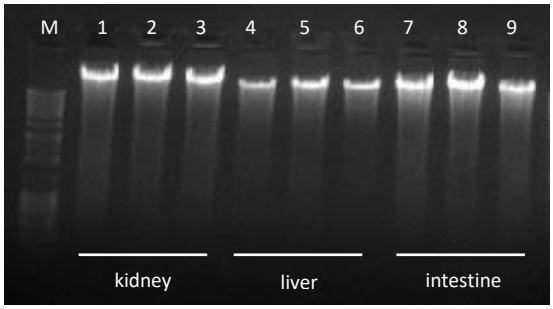


Figure 1. Integrity and quantification of extracted genomic DNA from fresh **human whole blood** samples using NZYtech's NZY Tissue, Blood & Cells gDNA Isolation Kit. Four independent whole blood samples were processed using two different kits: MB517 (green, NZY Tissue, Blood & Cells gDNA Isolation Kit) and MB135 (blue, NZY Tissue gDNA Isolation Kit, NZYtech). The Integrity of genomic DNA extracted from samples 1-8 is shown on a 1% agarose gel.

A



B

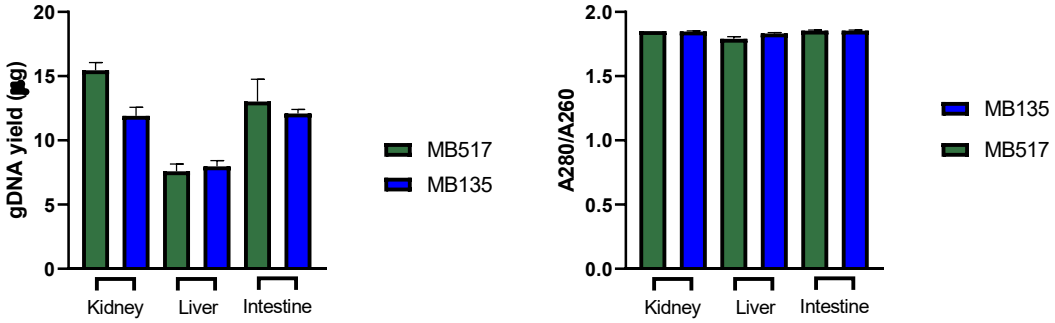


Figure 2. Genomic DNA isolation from **frozen tissue samples** using NZYtech's NZY Tissue, Blood & Cells gDNA Isolation Kit (MB517, green) and NZY Tissue gDNA Isolation Kit (MB135, blue). **A.** Integrity of genomic DNA samples extracted in triplicates from mouse kidney (1-3), mouse liver (4-6) and mouse intestine (7-9) using the NZY Tissue, Blood & Cells gDNA Isolation Kit. Samples are presented on 1% agarose gel. **B.** DNA yield and quality parameters obtained using both the NZY Tissue, Blood & Cells gDNA Isolation Kit (MB517, green) and NZY Tissue gDNA Isolation Kit (MB135, blue), following each supplier's recommended protocol. For the lysis step, MB517 extractions used a 30-min incubation at 56 °C, and MB135 extractions used a 1-h incubation at 56 °C. Tissue pieces were minced using a scalpel and homogenized in 180 µL of Buffer NT2/NT1 using an IKA Ultra-Turrax T10 basic.

Quality control

All components of NZY Tissue, Blood & Cells gDNA Isolation Kit are tested following the isolation protocol described above. The purification system must isolate 20-30 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 NT2/Supreme Proteinase K solution in accordance with the recommendations given above.
• **Inadequate Buffers preparation**
Check that Buffer NW4 concentrate was diluted with the correct volume of ethanol.
CLOGGED COLUMNS
• **Large amount of sample material**
Check if the amount of starting material used is recommended. Do not use more sample than recommended.
SUBOPTIMAL ELUTION
• **Low Elution Volume**
To improve elution efficiency, consider increasing the elution volume 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 NE2 (5 mM Tris / HCl, pH 8.5).
DEGRADED DNA
• **DNase Contamination**
Starting sample may have not been 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 NW3 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 incubating for 30 minutes at 37 °C.
• **Low A_{260}/A_{280} ratio**
Check that Buffer NW4 concentrate was prepared correctly. Ensure that Buffers NW3 and NW4 are used correctly. If the A_{260}/A_{280} ratio of the DNA is below 1.6, repeat the purification protocol.
• **Low A_{260}/A_{230} ratio**
The eluate probably contains carry-over of ethanol or salt. Ensure that centrifugation steps were done at ≥ 1 min at 13,000 *xg* to remove all traces of wash buffers. If necessary, repeat the centrifugation step.