# NZY Microbial gDNA Isolation Kit

**Catalogue number**
MB21702

**Presentation**
50 columns

## Description

NZY Microbial gDNA Isolation kits are designed for the simple and rapid small-scale preparation of highly pure genomic DNA from a wide variety of microbial samples. This kit ensures cell wall lysis using mechanical disruption instead of enzymatic methods. Difficult to lyse microbial samples such as yeast, gram-positive bacteria and spores can be lysed using NZY Microbial gDNA Isolation kit. NZYSpin Microbial Bead Tubes replace enzymatic lysis and by mechanical disruption they can lyse strong complex cell wall structures. *Bacterium* and yeast such as *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, *Bacillus subtilis* and *Escherichia coli* are examples of microbial organisms whose DNA can be isolated using this kit. NZY Microbial gDNA Isolation kit is optimized to isolate 5-25 µg of DNA from up to 30 mg wet weight of microbial pellet, depending on the type of sample.

## 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 | MB21702 (50 COLUMNS) |
|---|---|
| Buffer NML | 35 mL |
| Buffer NMW1 | 30 mL |
| Buffer NMW2 (concentrate) | 6 mL |
| Buffer NME | 12 mL |
| Proteinase K (liquid) | 600 µL |
| NZYSpin Microbial Bead Tubes | 50 |
| NZYSpin Microbial Columns (light green rings) | 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

## Specifications

**Expected genomic DNA Yield:** This protocol was designed for purification up to 25 µg of pure DNA (from up to 30 mg wet weight of microbial pellet) 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 NML and NMW1 chaotropic salts which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste. Wear gloves and goggles when using this kit.

**Procedures before starting**

Reagents Preparation

- Buffer NMW2: add 24 mL of 96-100% ethanol to the Buffer NW2 bottle.
- Preheat Buffer NME at 70 °C.

**Procedure**

**1. Sample preparation**

Harvest cells by centrifugation in a microcentrifuge tube and discard supernatant.

Ressuspend cells (up to 40 mg of wet weight) in 100 µL of Elution Buffer NME.

**2. Lysis of sample**

Transfer the resulting suspension into a NZYSpin Microbial Bead Tube and add 40 µL of Buffer NML. Add 10 µL of Proteinase K (liquid) and close the tube.

***Note:*** *The use of vortex is not necessary here.*

Put the NZYSpin Microbial Bead Tube on a swing mill or similar device and agitate.

***Note:*** *Optimal speed and agitation duration depends on the machine used. On a Retsch Schwingmuhle MM200, MM300, MM400, 4 min at 30 Hertz is adequate for E. coli and 12 min for B. subtilis.*

Centrifuge the NZYSpin Microbial Bead Tube for 30 s at 11,000 × *g*.

**3. DNA binding**

Add 600 µL of Buffer NML and mix in the vortex for 3 s.

***Note:*** *Glass beads should be resuspended*.

Then, centrifuge for 30 s at 11,000 × *g*. Transfer the supernatant onto the NZYSpin Microbial Column and centrifuge for 30 s at 11,000 × *g*. Discard the collection tube with the flow through and place the column in a new collection tube.

**4. Wash silica membrane**

Add 500 µL of Buffer NMW1 to the NZYSpin Microbial column. Centrifuge for 30 s at 11,000 × *g*. Discard flow-through and place the column back into the collection tube.

Add 500 µL of Buffer NMW2 (make sure ethanol was previously added) to the NZYSpin Microbial column and centrifuge for 30 s at 11,000 × *g*. Discard flow-through.

**5. Dry silica membrane**

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

**6. Elute DNA**

Place the NZYSpin Tissue column into a clean microcentrifuge tube and add 100 µL of Buffer NME (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 NME (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 Microbial gDNA Isolation kit are tested following the isolation protocol described above. The purification system must isolate 5-25 µg of gDNA/column, depending on the source of the tested samples.

For life science research only. Not for use in diagnostic procedures.

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