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NZY Microbial gDNA Isolation kit

Catalogue number: MB21702, 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 of the type of sample.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (18-25 °C) and are stable till the expiry date. Before use, add 24 mL of ethanol (96-100%) to buffer NMW2 bottle. 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.

System Components

Component	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

Standard protocol for isolating genomic DNA

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

To remove residual wash buffer, centrifuge the column for 30 s at 11,000 × *g*.

6. Elute DNA

Place the NZYSpin Microbial column into a clean microcentrifuge tube and add 100 µL of Buffer NME (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 ×*g* for 30 s to elute DNA. The genomic DNA can be stored at 4 °C or, preferably, at -20 °C.

Quality control assay

Functional assay

All components of NZY Microbial gDNA Isolation kit are tested following the isolation protocol described above. The purification system should isolate 5-25 µg of gDNA/column, depending on the source of the tested samples.

V1901

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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