NZY Soil gDNA Isolation kit

Catalogue numbers: MB21802, 50 columns

Description

NZY Soil gDNA Isolation kits are designed for the simple and rapid small-scale preparation of highly pure genomic DNA from a wide variety of soil microorganisms, such as bacteria, archaea, fungi, and algae in soil, sludge and sediment samples. This kit is suitable for samples from forest, bog, farmland, grassland and stool samples. The samples are mechanically disrupted using ceramic beads. Proteins and other PCR inhibitors in solution are precipitated with Buffer NSL3 followed by centrifugation with the ceramic beads. Any residual humic substances and other PCR inhibitors are efficiently removed in the washing steps. High yields with excellent purity from all types of samples are possible due to the combination of Buffer NSL1 and Buffer NSL2 with the additive NS enhancer.

NZY Microbial gDNA Isolation kit is optimized to isolate 2-10 µg of DNA from up to 500 mg of soil or sediment.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (18-25 °C) and are stable for at least one year. For longer storage, keep all contents at 4 °C.

Before use, add 100 mL of 100% molecular biology grade ethanol to each bottle of buffer NSW2.

Buffers NSB and NSW1 contain guanidine hydrochloride and/or detergents. Wear gloves and goggles when using this kit. Buffers NML and NMW1 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

System Components

Component	50 columns
Buffer NSL1	60 mL
Buffer NSL2	60 mL
Buffer NSL3	10 mL
NS Enhancer	10 mL
Buffer NSB	60 mL
Buffer NSW1	30 mL
Buffer NSW2 (conc.)	25 mL
Buffer NSE	13 mL
NZYSpin Soil Bead Tubes	50
NZYSpin Soil Inhibitor Removal Columns	50
NZYSpin Soil Columns	50
Collection Tubes (2 mL)	50
Collection tubes (2 mL, lid)	50

Standard protocol for isolating genomic DNA

1. Sample preparation

Place 250-500 mg of sample material in a NZYSpin Soil Bead Tube.

Note: *Do not exceed the 1 mL mark*

Add 700 µL of Buffer NSL1 or Buffer NSL2.

Note: *Due to the wide variety of samples that can be used, it is impossible to predict which buffer is best. It is wise to test both buffers and analyse which one presents best results.*

- If the sample material is very dry and soaks too much lysis buffer, fill the tube up to 1.5 mL mark.

- If the sample material is very wet, remove the excess liquid before the addition of lysis buffer.

2. Sample lysis (Buffer NFL)

Add 150 µL of NS Enhancer to the tube and close the cap.

Put the NZYSpin Soil Bead tubes horizontally in a vortex (by taping or using an adapter). Vortex at full speed for 5 min at room temperature.

3. Contaminants precipitation

To eliminate the foam caused by the detergent, centrifuge for 2 min at 11,000 g.

After this, add 150 µL of Buffer NSL3 and vortex for 5 s. Proceed by incubating for 5 min at 0-4ºC. Centrifuge for 1 min at 11,000 g.

4. Lysate filtration

Load up to 700 µL of the supernatant from the previous step into the filter of a NZYSpin Soil Inhibitor Removal Column (red ring) placed in a Collection tube (2 mL, lid).

Centrigfuge at 11,000 g for 1 min and discard the NZYSpin Soil Inhibitor Removal Column.

Note: *If the sample volume exceeds 700 µL, transfer the NZYSpin Soil Inhibitor Removal Column to a new collection tube and load the excess supernatant. After this, combine the flow-throughs.*

If a pellet is present in the flow-through, transfer the supernatant to other collection tube.

5. DNA Binding

To adjust binding conditions, add 250 µL of Buffer NSB and vortex for 5 s.

Load 550 µL of the sample into a NZYSpin Soil Column (green ring) placed in a collection tube.

Centrifuge at 11,000 g for 1 min. Discard the flow-through and put the column in the collection tube.

Load the remaining sample and repeat the process.

6. Silica membrane washing and drying

Add 500 µL Buffer NSB to the NZYSpin Soil Column, centrifuge for 30 s at 11,000 g and discard flow-through.

Add 550 µL of Buffer NSW1 to the column, centrifuge for 30 s at 11,000 g and discard flow-through.

After this, add 700 µL Buffer NSW2 to the column, close the lid and vortex for 2 s, followed by a centrifugation at 11,000 g for 30 s.

Discard flow-through and repeat the previous step for a more efficient wash of the column. In order to dry the silica membrane, centrifuge for a further 2 min at 11,000 g.

7. DNA Elution

Place the NZYSpin Soil column into a clean microcentrifuge tube and add:

- 30 µL Buffer NSE if a high concentration of DNA is required;
- 50 µL Buffer NSE for a recovery with medium concentration and yield;
- 100 µL Buffer NSE for a high yield.

With the lid open, incubate for 1 min at room temperature. Close the lid and centrifuge at 11,000 g for 30 s.

The genomic DNA can be stored at 4 °C or, preferably, at -20 °C.

Quality control assay

Functional assay

All components of NZY Soil gDNA Isolation kit are tested following the isolation protocol described above. The purification system must isolate 2-10 µg of gDNA/column, depending on the source of the tested samples.

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Certificate of Analysis

Test	Result
Functional assay	Pass

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