MB177_IFU_EN_V2402

NZY Plant/Fungi gDNA Isolation Kit

Catalogue number
MB17701

Presentation
50 columns

Description

NZY Plant/Fungi gDNA Isolation kits are designed for the simple and rapid small-scale preparation of highly pure genomic DNA from plant tissues and fungi samples. The method is spin column silica-based and requires no phenol or chloroform extraction. This kit uses two optimized lysis buffers systems based on the established CTAB and SDS methods. NZY Homogenization columns are included in this kit for conveniently clearing the crude lysates. The clear flow-through is mixed with binding Buffer PN for optimal binding of DNA to the silica membrane. Then, the DNA is selectively absorbed into the NZYSpin Plant Column and others impurities such as proteins and salts are removed during the washing steps. The eluted genomic DNA has a A_{260}/A_{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 Plant gDNA Isolation Kit is optimized to isolate up to 30 µg of DNA from up to 100 mg (wet weight) or 20 mg (dry weight) of plant/fungi samples, depending of the plant/fungi species. We suggest not using more than the recommended starting material to prevent reductions in yield and purity.

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	MB17701 (50 COLUMNS)
Buffer PNL1	22 mL
Buffer PNL2	20 mL
Buffer PNL3	5 mL
Buffer PN	25 mL
Buffer PNW1	22 mL
Buffer PNW2 (concentrate)	25 mL
Buffer PNE	7 mL
RNase A (lyophilized)	6 mg
NZYSpin Homogenization columns (purple rings)	50
NZYSpin Plant columns (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 mixer
- Disruption and homogenization equipment's

Specifications

Expected genomic DNA Yield: This protocol was designed for purification up to 30 µg of pure DNA (from up to 100 mg of wet weight or up to 20 mg of dry weight) with an A_{260}/A_{280} ratio between 1.7 and 1.9.

Columns type: silica membrane technology

Elution Volume: 50-100 µL

Standard Protocol

Recommendations before starting

- Buffers PNL1, PNL2 and PN may form a precipitate when stored at low temperatures, if necessary, dissolve the precipitate by warming the solutions at 37 °C.
- Buffers PNL1, PNL2, PN and PNW1 contain guanidine hydrochloride and/or detergents like CTAB or SDS. Wear gloves and goggles when using this kit. DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Procedures before starting

Reagents Preparation

- Buffer PNW2: Add 100 mL of 96-100%) ethanol to Buffer PNW2 bottle.
- RNase A: Add 0.6 mL of water to the RNase A vial. Store the RNase A solution at 4 °C for up to 3 months. For longer storage (up to 1 year), the RNase A solution should be divided into small aliquots and stored at -20 °C.

Procedure

NZY Plant/Fungi gDNA Isolation Kit includes two different lysis buffers for optimal results with most common plant species. Please, see Table 1 (in appendix section) for choosing the optimal lysis buffer system for your individual plant/fungi sample. Please follow the procedures A (Plant tissues) or B (Fungi tissues) according with your starting material.

A. Genomic DNA from Plant

1. Sample preparation

Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material. Proceed with cell lysis using Buffer PNL1 (step 2a) or Buffer PNL2 (step 2b).

2a. Cell Lysis (Buffer PNL1)

Transfer the resulting powder to a new tube and add 400 µL Buffer PNL1. Mix thoroughly by vortex.

Note: *If the samples are difficult to lyse, additional Buffer PNL1 can be added. Note that the volumes of RNase A (step 2a) and Buffer PN (step 4) must be increased proportionally.*

Add 10 µL of RNase A solution and mix sample thoroughly. Incubate the suspension for 10 min at 65 °C.

Note: *For some plant samples, it might be advantageous to increase the incubation time to 30-60 min.*

Proceed with step 3.

2b. Cell Lysis (Buffer PNL2)

Transfer the resulting powder to a new tube and add 300 µL Buffer PNL2. Mix thoroughly by vortex.

Note: *If the samples are difficult to lyse, additional Buffer PNL2 can be added. Note that the volumes of RNase A, Buffer PNL3 (step 2b) and Buffer PN (step 4) must be increased proportionally*.

Add 10 µL of RNase A solution and mix sample thoroughly. Incubate the suspension for 10 min at 65 °C.

Note: *For some plant samples, it might be advantageous to increase the incubation time to 30-60 min.*

Add 75 µL of Buffer PNL3 and mix sample thoroughly. Incubate for 5 min on ice to precipitate SDS completely.

Proceed with step 3.

3. Clarification of Crude Lysate

Transfer the mixture from step 2 into a NZYSpin Homogenization column (purple ring) placed in a new 2 mL collection tube. Centrifuge for 2 min at >11,000 × *g*, collect the clear flow-through and discard the NZYSpin Homogenization column.

Notes:

- *If not, all liquid has passed the filter, repeat the centrifugation step.*
- *If insoluble particles are visible in the flow-through, transfer the clear supernatant to a new 1.5 mL microcentrifuge tube. Alternatively, centrifuge the crude lysate for 5 min at full speed and transfer the supernatant to a new microcentifuge tube or pass the precleared supernatant through the NZYSpin Homogenization column to remove the insoluble particles completely*.

4. Adjust DNA Binding Conditions

Add 450 µL of Buffer PN and mix thoroughly by pipetting up and down for 5 times.

5. DNA Binding

Place a NZYSpin Plant column (green ring) into a new collection tube and load 700 µL of the sample (maximum loading capacity of NZYSpin Plant column).

Centrifuge for 1 min at > 11,000 x *g* and discard flow-through. For higher sample volumes repeat the loading step.

6. Wash Silica Membrane

Add 400 µL of Buffer PNW1 to the NZYSpin Plant column. Centrifuge for 1 min at > 11,000 x *g*. Discard flow-through and place the column back into the collection tube.

Add 700 µL of Buffer PNW2 (make sure ethanol was previously added) to the NZYSpin Plant column and centrifuge for 1 min at > 11,000 x *g*. Discard flow-through.

Add another 200 µL of Buffer PNW2 to the NZYSpin Plant column and centrifuge for 2 min at > 11,000 x *g* to remove wash buffer and dry the silica membrane completely.

7. Elute DNA

Place the NZYSpin Plant Column into a clean microcentrifuge tube and add 50 µL of Buffer PNE directly in the membrane column (preheating of elution buffer to 65 °C may improve yield). Incubate 2-5 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 × 50 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 30 to 40 µL. This approach typically yields around 60 – 80% of the bound nucleic acids, producing highly concentrated eluates.

Additionally, you can substitute Buffer PNE (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.

B. Genomic DNA from Fungi

1. Sample preparation

Wash 50-200 mg mycelium (fresh weight) or material from fruiting body of macro fungi in ethanol. Mycelium can be obtained from a liquid culture or scraped off (with or without agar) from the surface of a solid medium. Cover sample completely with ethanol and mix carefully. Short washing in ethanol is sufficient in most cases, although incubation overnight sometimes increases DNA yield.

Remove the ethanol by pipetting and squeezing the mycelium.

2. Cell lysis

Place the sample into a 1.5 mL microcentrifuge tube. Add 150 mg siliconized glass beads or sea sand and 200 µL of Buffer PNL1. Homogenize sample using a micro pistil and vortex regularly. Add additional 100 µL of Buffer PNL1 and continue to homogenize the sample.

Note: *If the samples cannot be handled easily, additional Buffer PNL1 can be added. Note that the volume of Buffer PN (step 4) must be increased proportionally. If the samples are rich in RNA or protein, we recommend adding 10 µL of RNase A and/or Proteinase K (5-10 mg/mL stock solution) to the PNL1 lysis solution to minimized contaminants.*

Incubate for 10 min at 65 °C. For some fungi, it might be advantageous to increase the incubation time to 30-60 min.

Add 100 µL chloroform, vortex for 10 seconds and separate phases by centrifugation for 15 min at 20,000 × *g*. Pipette the top aqueous layer into a new 1.5 mL microcentrifuge tube.

Proceed with section A, step 3.

Quality control assay

All components of NZY Plant/Fungi gDNA Isolation Kit are tested following the isolation protocol described above. The purification system must isolate 10-30 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.
• **Inadequate Buffers preparation**
Check that Buffer PNW2 concentrated was diluted with correct volume of ethanol.
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 repeating the elution step up to three times. Ensure that the elution buffer is preheated to 65 °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 PNE (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.
LOW 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 PNW2 concentrated was prepared correctly. Ensure that Buffers PNW1 and PNW2 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 *x g* 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 Plant/Fungi gDNA Isolation Kit.

Appendix

Table 1. Plant species tested with NZY Plant/Fungi gDNA Isolation Kit.

PLANT SPECIES	PLANT TISSUE/ORGAN	LYSIS BUFFER SUCCESSFULLY TESTED	
		PNL1	PNL2
Abies alba (fir)	Needle	Ok	Ok
Amorphorphallus titanium	Leaf	Ok	Not tested
Apium graveolens (celery)	Corm	Ok	Ok
Arabidopsis thaliana	Leaf	Ok	Ok
Boreava orientalis	Leaf, herbarium sample	Ok	Ok
Cleisostoma racemiferum	Inflorescence rachis, silica-gel dried	Ok	Not tested
Doritis pulcherrima	Leaf, silica-gel dried	Ok	Not tested
Eichornia azurea	Leaf	Ok	Not tested
Encephalartos natalensis	Leaf	Ok	Not tested
Galium aparine	Leaf	Ok	Ok
Hordeum sp. (barley)	Leaf	Ok	Ok
Isatis kotschyana	Leaf, herbarium sample	Ok	Ok
Laurus azorica (laurel)	Leaf	Ok	Not tested
Lupinus sp. (lupin)	Leaf	Ok	Ok
Lycopersicon esculentum (tomato)	Stem	Ok	Ok
Myagrum perfoliatum	Leaf, herbarium sample	Ok	Ok
Oryza sativa (rice)	Leaf	Ok	Ok
Persea feru./caerulea	Leaf	Ok	Not tested
Pteridium sp.	Leaf	Ok	Not tested
Pterocarya fraxiniofolia	Leaf	Ok	Not tested
Rosa sp. (rose)	Leaf	Ok	Ok
Rubus fruticosus (blackberry)	Leaf	Ok	Ok
Sameraria nummularia	Leaf, herbarium sample	Ok	Ok
Secale sp. (rye)	Leaf	Ok	Ok
Stereochilus sp.	Leaf, silica-gel dried	Ok	Not tested
Tauscheria lasiocarpum	Leaf, herbarium sample	Ok	Ok
Trachycarpus takil	Leaf	Ok	Not tested
Trichoglottis sp.	Leaf, silica-gel dried	Ok	Not tested
Triticum aestivum (wheat)	Leaf	Ok	Ok
Vigna radiate (mung bean)	Root	Ok	Ok
Zea mays (maize)	Leaf	Ok	Ok
Zea mays (maize)	Grain, dried, ground coarsely	Ok	Ok
Fungal mycel (not specified)		Ok	Not tested
Green algae (not specified)		Ok	Not tested