dNEAT PLANT DNA EXTRACTION KIT

PURK-PLN-024

Introduction

dNEAT Plant DNA Extraction Kit combines a modified CTAB-based lysis method with silica column purification technology for the efficient isolation of high-quality genomic DNA from a wide range of plant tissues and fungal samples. The protocol is designed to address common challenges associated with plant DNA extraction, including the presence of polysaccharides, polyphenols and secondary metabolites that can interfere with downstream applications.

The extraction procedure utilises a CTAB-based buffer system to ensure effective cell lysis and solubilisation of nucleic acids, while facilitating the removal of complex carbohydrates and phenolic compounds. The kit incorporates a less toxic substitute for chloroform (Buffer BDP), improving laboratory safety while maintaining efficient phase separation and contaminant removal. Purification is achieved through an alcohol-free, high-concentration guanidine salt-mediated filtration system, allowing selective binding of DNA to the column matrix and efficient elimination of polysaccharides, polyphenols, proteins, metabolites and RNA. The resulting DNA is of high purity and integrity, suitable for sensitive molecular biology applications.

Contents

Components	24 rxn
Buffer PAL	20 ml
Buffer BDP	20 ml
Buffer GWP	20 ml
Buffer SW2	30 ml
Elution buffer	10 ml
DNA Spin Column	24 pcs
Collection Tube	24 pcs

Storage

This product can be stored at room temperature (15~25˚C) for 18 months. Buffer PAL may precipitate at low temperatures, which can be dissolved by incubating at 55ºC and mixing thoroughly. The product uses PET reagent bottles, which should not be subjected to high-temperature, high-pressure sterilization or direct incubation above 55ºC.

Product use limitation

This product is developed and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration for humans or animals.

Protocol

A. ROUTINE SAMPLES

1. Sample Grinding

Grind plant or fungal material into a fine powder in liquid nitrogen.

Transfer:

- 50–150 mg fresh/frozen sample, or
- 15–40 mg dried sample

into a 2 ml centrifuge tube.

Important:

- Correct sample amount is essential. Excess material may block the column and reduce yield and purity.
- Due to variability in DNA and metabolite content, it is recommended to start with 50 mg fresh or 15 mg dried material and adjust as needed.
- For mucilage-rich samples, do not exceed 30–50 mg fresh weight.

Increasing yield:

- Column binding capacity: 20 µg DNA.
- If DNA content is low, increase sample amount 2–3 fold and proportionally increase Buffer PAL and Buffer BDP. Purify using multiple column passes if necessary.

2. Lysis

Immediately add 700 µl Buffer PAL (preheated to 65°C).

Vortex thoroughly to disperse the sample.

Incubate at 65°C for 15–30 minutes, mixing 2–3 times during incubation.

Optional:

- Add β-mercaptoethanol to Buffer PAL to a final concentration of 2% to enhance antioxidant capacity.
- Because of its strong odour, most samples do not require β-mercaptoethanol.

Important:

Do not add PVP-40 at this stage for routine samples.

3. Organic Extraction

Add 700 µl Buffer BDP (or chloroform).

Vortex 15 seconds.

Centrifuge at 12,000 × g for 5 minutes at room temperature.

For samples rich in polyphenols or starch:

Perform an additional phenol–chloroform extraction before this step.

Safety note:

Buffer BDP contains bromochloropropane (less toxic than chloroform). In case of skin contact, remove contaminated clothing immediately, rinse thoroughly with water and seek medical advice.

4. Adjustment of Binding Conditions (High-Salt Mediation)

Transfer 600 µl supernatant to a new tube.

Add 600 µl Buffer GWP and mix by inversion (6–8 times).

Under guanidine conditions:

- The column binds DNA but not RNA (RNase treatment is generally unnecessary).
- Maximum binding capacity under these conditions: 10 µg DNA.
- Excess DNA will pass through.

High-salt conditions remove pigments, polysaccharides, proteins and secondary metabolites.

If DNA content exceeds 10 µg (alcohol-mediated method):

1. Transfer 600 µl supernatant to a new tube.
2. Add 10 µl RNase A (self-prepared). Incubate 10 minutes at room temperature.
3. Add 300 µl Buffer GWP and 600 µl absolute ethanol.
4. Mix 15–30 times by inversion.
5. Proceed to Step 5.

5. Column Purification

Place the spin column in a collection tube.

Load half of the mixture onto the column.

Centrifuge at 10,000 × g for 1 minute.

Discard filtrate.

Reload remaining mixture and centrifuge again.

6. Removal of Proteins and RNA

Discard filtrate.

Add 400 µl Buffer GWP.

Centrifuge at 10,000 × g for 1 minute.

7. Desalting

Discard filtrate.

Add 750 µl Buffer SW2.

Centrifuge at 10,000 × g for 1 minute.

If DNA concentration is below 50 ng/µl, divide Buffer SW2 into two washes of 500 µl each to improve the A260/230 ratio.

8. Dry Spin

Discard filtrate.

Centrifuge at 10,000 × g for 1 minute to remove residual ethanol.

9. Elution

Transfer column to a clean 1.5 ml tube.

Add 50 µl Elution Buffer (preheated to 65°C) directly to the membrane centre.

Incubate 3 minutes at room temperature.

Centrifuge at 10,000 × g for 1 minute.

10. Re-elution

Repeat with an additional 50 µl preheated Elution Buffer.

Incubate 3 minutes.

Centrifuge at 10,000 × g for 1 minute.

11. Storage

Discard the column.

Store DNA at −20°C.

B. DIFFICULT-TO-EXTRACT PLANT SAMPLES

For samples with low nucleic acid content or high levels of inhibitors:

Add to Buffer PAL:

- 50 µl of 40% PVP-40 per 1 ml Buffer PAL
- 20 µl β-mercaptoethanol per 1 ml Buffer PAL

(Store this mixture at room temperature for up to one month.)

1. Grinding and Lysis

Grind sample in liquid nitrogen.

Add preheated (65°C) PAL/PVP-40 mixture immediately.

Vortex thoroughly.

Incubate at 65°C for 20 minutes, mixing 2–3 times.

Buffer usage guideline:

- 0.7 ml Buffer PAL per 100 mg fresh or 20 mg dried sample.
- Sample amount may be increased to 200–500 mg if needed; adjust Buffer PAL proportionally.

2. Organic Extraction

Add equal volume Buffer BDP or chloroform.

Vortex 15 seconds.

Centrifuge at 10,000 × g for 5 minutes.

If total volume exceeds 2 ml:

- Use a 5–15 ml tube.
- Centrifuge at 4,000–5,000 × g for 15 minutes.

Optional phenol–chloroform extraction may be added for polyphenol- or starch-rich samples.

3. Precipitation Enrichment

Transfer supernatant to a new tube.

Add 0.7 volumes isopropanol.

Invert gently 15–20 times.

Centrifuge at 10,000 × g for 5 minutes.

Discard supernatant and retain pellet.

If volume > 2 ml:

- Use 5–15 ml tube.
- Centrifuge at 4,000–5,000 × g for 10 minutes.

Notes:

- Silky/fibrous precipitate indicates abundant DNA.
- Flocculent precipitate suggests high polysaccharide content (repeat phenol–chloroform extraction).
- If no visible precipitate forms, incubate at −20°C overnight before centrifugation.

4. Dissolution

Remove residual liquid.

Add 200 µl Elution Buffer or sterile water.

Incubate at 65°C for 10–15 minutes to dissolve DNA.

Crude genomic DNA from other methods may also be purified:

- Adjust volume to 250 µl with water.
- Proceed to Step 5.

5. Column Purification

Add 400 µl Buffer GWP.

Mix by inversion.

Follow Steps 5–11 of Procedure A.

If DNA exceeds 10 µg:

- Add 0.2 ml isopropanol.
- Mix and proceed to column purification.

For polysaccharide-rich samples:

- Incubate at 50°C for 10 minutes.
- Pipette repeatedly to disperse gel-like material before purification.

OD Value Measurement and Yield

A260/280 ratio: 1.70–1.90

A260/230 ratio: 1.1–2.4

If nucleic acid concentration is below 50 ng/µl, lower A260/230 values may still be acceptable.