RUO

REF	G2MBR4-0654	50 Tests
	G2MBR4-0655	250 Tests

SPINXT Plant DNA Extraction Kit

Intended Use

This protocol is suitable for extracting DNA from fresh/-frozen/dry plant samples. Purified DNA includes genomic DNA and chloroplast DNA.

Intended User

The assay is intended to be performed by a laboratory professional in research laboratory.

Test Principal

The SpiNXT Plant DNA Extraction Kit is a laboratory tool used to isolate and purify DNA from plant cells. Plant tissue is ground into a fine powder to release the cellular contents. Lysis buffer is added to disrupts cell membranes and releases the DNA, inactivates DNAses, and helps to solubilize DNA. The DNA is bound to a silica membrane, separating it from other cellular components. Impurities are removed through a series of washes. The purified DNA is released from the silica membrane. The extracted DNA is measured for concentration and purity. These components work together to enable efficient and effective extraction of high-quality DNA from plant tissues, suitable for downstream applications like RT-PCR, qRT-PCR, and DNA sequencing. This process allows researchers to study gene expression, analyze plant responses to environmental stressors, and identify potential genetic markers for crop improvement.

Summary

SpiNXT Plant DNA Extraction Kit offers a simple and rapid extraction of genomic DNA based on silica membrane based spin column technology from different plant species and tissues such as Leaves of Chickpea, Wheat, Soya bean, Marigold leaves, Rice, Chickpea green seed, stems, Tulsi leaves with sample sizes of 10-50 mg tissue. Purified DNA can be used directly for PCR, quantitative PCR, Southern Blot, hybridization, transgenesis detection, restriction enzyme digestion, and Southern blotting etc.

Equipment and Reagents Required but Not Provided

- 2-Mercaptoethanol (βME)
- Water bath or heat block
- Micropipettes
- Disposable barrier (Filter) pipette tips
- 1.5 ml micro-centrifuge tubes
- Table top micro-centrifuge
- Ethanol (96-100%)
- Personal protection equipment (Aprons, gloves, goggles etc.)
- Mortar and pestle

Storage and Stability

- The kit has a shelf life of 18 months from the date of manufacture.
- The test kit is stable until the expiration date marked on the kit box and/or the packaging of individual components when stored at room temperature (15-25°C).
- If precipitate forms in any of the reagents of the kit, warm at 55°C to dissolve.

⚠Instructions Before Use

- Pre heat buffer D-EB at 60°C as per required volume.
- Switch on the water bath at 60°C before starting of the experiment.
- Use preheated Buffer AE for efficient DNA yield.
- Use sterile 1.5 ml microcentrifuge tubes.
- Dilute Buffer PW1/PW2 with an appropriate amount of absolute ethanol as shown on the label.
- Add 2-mercaptoethanol: Add 3% 2-mercaptoethanol to Buffer D-EB to improve the antioxidant capacity of lysate.

Reagents Provided

Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0654
RNaseA (10mg/ml)	G2MBR3-1489-1	1 X 250 µl
Buffer D-EB	G2MBR3-1490-1	1 X 55 ml
Buffer PW1	G2MBR3-1491-1	1 X 20 ml
Buffer PW2	G2MBR3-1492-1	1 X 20 ml
Buffer AE	G2MBR3-1493-1	1 X 10 ml

Consumables Provided

Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0654
Mini Column	1 X 50 Nos.
Collection Tube	1 X 50 Nos.

Reagents Provided

Table 2a. (For 250 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0655
RNaseA (10mg/ml)	G2MBR3-1489-2	1 X 1.25 ml
Buffer D-EB	G2MBR3-1490-2	1 X 260 ml
Buffer PW1	G2MBR3-1491-2	1 X 90 ml
Buffer PW2	G2MBR3-1492-2	2 X 45 ml
Buffer AE	G2MBR3-1493-2	1 X 30 ml

Consumables Provided

Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0655
Mini Column	2 X 125 Nos.
Collection Tube	2 X 125 Nos.

Before using Buffer PW1 and Buffer PW2 for the first time, add the appropriate volume of molecular biology grade ethanol (96–100%) as indicated on the bottle and shake thoroughly. Buffer PW1 and Buffer PW2 are stable for at least eighteen months after the addition of ethanol, when stored at room temperature (15–25°C) in closed condition.

Important Notes

- Sample collection and storage shall be performed according to the recommended procedure.
- Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA yield.
- Use of poor-quality starting material may affect yield of purified DNA.

Protocol

DNA Purification from Plant sample

1) Grind wet ≤50mg or dry ≤20mg of plant samples in to powder form using mortar and pestle in presence of 1ml preheated Buffer D-EB at 60°C. 3% of 2-Mercaptoethanol (βME) need to be added into Buffer D-EB immediately before use.

2) Transfer the sample mixture into 1.5 ml micrcentrifuge tube and incubate at 60°C for 20 min followed by inverting the tube in every 5 min.

3) To get clear plant lysate, centrifuge the tube at 16,000x*g* for 5 min and transfer the supernatant to clean 1.5 ml microcentrifuge tube.

4) Add 5 µl RNAseA (10mg/ml) into the solution followed by incubation at 37°C for 15 min.

5) Add 0.5 volume of (96-100%) Ethanol. Vortex for 5 sec.

6) Keep at room temperature for 2 min.

7) Transfer the mix on mini column and Centrifuge the sample at 16,000x*g* for 1 min. Discard collection tube with the flow through.

8) Transfer the column into a fresh collection tube (2 ml) and wash with 700 µl PW1. Centrifuge at 16,000x*g* for 1 min. Discard the flow through.

9) Place the column back into the collection tube and wash with 700 µl Buffer PW2. Centrifuge at 16,000x*g* for 1 min. Discard the flow through.

10) Repeat step 9.

11) Transfer the column back into the collection tube and give a dry spin at 20,000x*g* for 2 min.

12) Place the column into a fresh 1.5 ml microcentrifuge tube.

13) Elute the DNA in 100 µl Nuclease Free Water or Buffer AE provided in the kit; give 3 min incubation before centrifugation at 6,000x*g* for 1 min.

14) Store the DNA sample (s) at 4°C for immediate use or −20°C for long-term storage. If the lab is equipped with a −80°C freezer, we recommend storing at −80°C for long-term storage in order to preserve the quality.

Optional steps

- To increase final yield, please add equal volume of chloroform: Isoamyl alcohol (1:1) with clear plant lysate after step 3. Vortex for 5 sec then centrifuge the sample for 5 min at 16,000x*g* and use upper aqueous layer.
- **Note:** All the centrifugation steps need to be carried out at 4°C.

Symbols for use in the labeling	
Symbols	**Definition**
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <N> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED

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