RUO

REF	G2MBR4-0656	50 Tests
	G2MBR4-0657	250 Tests

SPINXT Plant RNA Extraction Kit

Intended Use

This protocol is suitable for extracting RNA from fresh/ frozen/dry plant samples.

Intended User

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

Test Principal

The SpiNXT Plant RNA Extraction Kit is a laboratory tool used to isolate and purify RNA (ribonucleic acid) 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 RNA, inactivates RNases, and helps to solubilize RNA. The RNA is bound to a silica membrane, separating it from other cellular components. Impurities are removed through a series of washes. The purified RNA is released from the silica membrane. The extracted RNA is measured for concentration and purity. These components work together to enable efficient and effective extraction of high-quality RNA from plant tissues, suitable for downstream applications like RT-PCR, qRT-PCR, and RNA sequencing. This process allows researchers to study gene expression, analyze plant responses to environmental stressors, and identify potential genetic markers for crop improvement.

Summary

The SpiNXT Plant RNA Extraction Kit is designed for efficient and rapid isolation of high quality intact RNA from a wide variety of plant species such as leaves of Chickpea, Wheat, Soya bean, Marigold leaves, Rice, Chickpea green seed, stems, Tulsi leaves and tissue types with sample sizes of 10-50mg tissue. The kit utilizes a convenient spin column format using silica based membrane technology, eliminating the use of toxic phenol/chloroform extractions, or time consuming alcohol precipitation. The purified high-quality RNA can be used for all the down-stream applications such as rnd point/Real Time, Northern Blotting other RNA based analysis, restriction enzyme digestion, 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.)
- Disposable gloves
- 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 R-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 RNA yield.
- Use sterile 1.5 ml micro-centrifuge 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 R-EB to improve the antioxidant capacity of lysate.

Kit Contents

Reagents Provided
Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0656
Buffer R-EB	G2MBR3-1320-1	1 X 60 ml
Buffer PW1	G2MBR3-1321-1	1 X 20 ml
Buffer PW2	G2MBR3-1322-1	1 X 20 ml
Buffer AE	G2MBR3-1323-1	1 X 10 ml

Consumables Provided
Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0656
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 G2MBR4-0657
Buffer R-EB	G2MBR3-1320-2	1 X 260 ml
Buffer PW1	G2MBR3-1321-2	1 X 90 ml
Buffer PW2	G2MBR3-1322-2	2 X 45 ml
Buffer AE	G2MBR3-1323-2	1 X 30 ml

Consumables Provided
Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0657
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 one year 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 RNA yield.
- Use of poor-quality starting material may affect yield of purified RNA.

Protocol

A. RNA Purification from Plant samples

1. Grind wet ≤50mg or dry ≤20mg of plant samples into powder form using mortar and pestle in presence of 1 ml preheated Buffer R-EB at 60°C. 3% of 2-Mercaptoethanol (βME) need to be added into Buffer R-EB immediately before use.
2. Transfer the sample mixture into 1.5ml micro-centrifuge tube, incubate at 60°C for 20 min. Mix during the incubation by gentle vortexing or 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 a clean 1.5 ml micro-centrifuge tube.
4. Add half volume of (96-100%) ethanol to the supernatant separated from step 3. Mix well by Gentle Vortex for 5 sec or gently inverting the tube.
5. Keep at room temperature for 2 min.
6. Transfer the mix on mini column and centrifuge the sample at 16,000x*g* for 1 min. Discard collection tube with the flow through.
7. Transfer the column into a fresh collection tube (2 ml) and wash with 700 µl PW1 and centrifuge at 16,000x*g* for 1 min. Discard the flow through.

8. Place the column back into the collection tube and wash with 700 µl Buffer PW2 and centrifuge at 16,000x*g* for 1 min. Discard the flow through.
9. Repeat step 8.
10. Transfer the column back into the collection tube and give a dry spin at 20,000x*g* for 2 min.
11. Place the column into a fresh 1.5 ml micro-centrifuge tube.
12. Elute the RNA in 100µl RNase/DNase Free Water or AE buffer provided in the kit, give 3 min incubation before centrifugation at 6,000x*g* for 1 min at room temperature.
13. Use the purified RNA in the downstream or store the extracted RNA sample (s) at -20°C for immediate use or -80°C for long-term storage in order to preserve the quality.

Optional step

DNAse I Treatment (Optional)

If DNA content of the sample is causing an obstruction/interfering in downstream applications of the purified RNA, an optional DNAse I step may be performed after the RNA elution (As per the manufacturer's instructions).

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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