Magnetic Plant RNA Kit

Cat.No.: HYC335

Product Description

This kit delivers a simple and high-throughput automated workflow for total RNA extraction from plant tissues. After physical disruption of plant samples, RNA in lysates binds to silica-coated magnetic beads under high-salt conditions. Contaminants such as proteins are washed off with wash buffers, followed by DNase I digestion to eliminate genomic DNA. Purified RNA is washed thoroughly and finally eluted into RNase-free water. Total RNA isolated by this kit features high purity, free of genomic DNA, protein and other impurities, and is compatible with diverse downstream applications including Real-Time RT-PCR, RT-PCR, Northern Blot, Dot Blot and in vitro translation.

Components

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DNase I (RNase Free)	4×1000U
10×Reaction Buffer	1 mL
Buffer PRL	60 mL
Buffer PA	40 mL
Buffer PGW1*	25 mL
Buffer RW2*	40 mL
RNase-Free Water	10 mL
Magbeads PN	2×1 mL

Storage

Store DNase I (RNase Free) and 10× Reaction Buffer at 2–8 °C. All other components should be kept at ambient temperature (10–30 °C).

Required Reagents

Anhydrous ethanol (freshly opened or dedicated for RNA extraction)

Instructions

Preparations

1. For newly opened kits, add the specified volume of anhydrous ethanol to Buffer PGW1 and Buffer RW2 respectively as labeled on each bottle prior to first use. Confirm ethanol supplementation before experiments. Tighten caps firmly after ethanol addition to prevent evaporation.
2. Wear disposable masks and gloves during the entire procedure, and replace gloves frequently. Only use RNase-free plastic consumables and pipette tips to avoid RNase contamination.
3. High-starch plant tissues (e.g., dry wheat seeds, dry corn seeds) will form gelatinous precipitates upon mixing with lysis buffer, and more gelatinous substances will accumulate with prolonged lysis time. This kit is not recommended for such samples; contact our technical support to obtain an alternative lysis buffer.
4. Fresh plant materials are recommended for RNA extraction. If extraction cannot be performed immediately, snap-freeze tissues in liquid nitrogen and store below −70 °C. Repeated freeze-thaw cycles must be avoided as they reduce RNA yield and integrity.
5. Instructions for DNase I (RNase Free): Add 500 µL RNase-free water to the cryovial containing DNase I (RNase Free). Mix by gentle tube inversion; do not vortex. The reconstituted DNase I stock concentration is 2 U/µL, store at −20 ± 5 °C.
6. Preparation of DNase I Working Mixture: Mix 52 µL RNase-free water, 8 µL 10× Reaction Buffer and 20 µL DNase I stock (2 U/µL) to prepare a total of 80 µL working mixture.

Sample Preprocessing

1. Plant Tissue Disruption

a. Grind 30–100 mg fresh or frozen plant tissue thoroughly into fine powder with liquid nitrogen. Add 600 µL Buffer PRL and vortex vigorously immediately for complete homogenization and lysis.

b. Load 30–100 mg fresh or frozen plant tissue, 600 µL Buffer PRL and one steel bead into a 2.0 mL centrifuge tube. Secure the tube on a tissue homogenizer for disruption. Centrifuge at 14,000 rpm for 5 min at 4 °C, then transfer all supernatant (approx. 500 µL) to a new user-supplied 1.5 mL centrifuge tube.

Note: Avoid aspirating suspended debris when collecting supernatant. Minor suspended particles are normal; extend centrifugation time if excessive debris is observed.

Manual Operation Workflow

1. (Continued from sample preprocessing) Add 350 µL Buffer PA and 20 µL Magbeads PN to the 1.5 mL tube and vortex to mix thoroughly. Incubate on a thermomixer with shaking at 1,700 rpm for 5 min at ambient temperature. Perform a brief spin-down, place the tube on a magnetic rack, and discard all supernatant once the liquid clears.
   Note: Fully resuspend Magbeads PN before use. Shake the bead stock thoroughly between sample additions when processing multiple specimens.
2. Remove the tube from the magnetic rack and add 500 µL Buffer PGW1 (confirm anhydrous ethanol has been added prior to use). Vortex to resuspend beads, then shake on a thermomixer at 1,700 rpm for 2 min at room temperature. Spin down briefly, set on the magnetic rack and discard supernatant after clarification.
3. Take the tube off the magnetic rack, add 80 µL DNase I working mixture and vortex to mix. Shake at 1,700 rpm on a thermomixer for 5 min at ambient temperature. Briefly spin down, place on the magnetic rack and discard clarified liquid.
4. Remove the tube from the magnetic rack, add 700 µL Buffer RW2 (verify anhydrous ethanol supplementation before use) and vortex to resuspend beads. Incubate with shaking at 1,700 rpm for 2 min at room temperature on a thermomixer. Spin down briefly, place on magnetic rack and discard supernatant once clear.
5. Repeat Step 4 once.
6. Air-dry beads at ambient temperature for 5–10 min to fully evaporate residual ethanol; stop drying when beads appear matte without cracking. Add 100 µL RNase-free water and homogenize by pipetting up and down or vortexing. Spin down briefly, then incubate on a thermomixer with shaking at 1,700 rpm and 65 °C for 10 min.
   Note: After discarding waste liquid, perform a quick spin-down, place the tube on the magnetic rack, and remove residual liquid with a 10 µL pipette tip.
7. Fix the tube on the magnetic rack until the solution clears, then transfer the eluted RNA to a new centrifuge tube. Store at −20 ± 5 °C for short-term use; store below −70 ± 10 °C if downstream experiments cannot be performed promptly.

8. (Optional) Supplement eluted RNA with RNase Inhibitor to a final concentration of 1 U/μL to effectively mitigate RNA degradation.

   Note: RNase Inhibitor is not supplied with this kit by default.