Spin Plant RNA Plus Kit

Cat.No.: HYC338

Product Description

This kit is suitable for RNA extraction from a wide range of plant samples. High-quality RNA can be efficiently isolated even from plant materials rich in polysaccharides and polyphenols. Applicable samples include rice leaves, wheat leaves, maize leaves, tobacco leaves, pine needles, ginkgo leaves, poplar leaves, pomegranate leaves, holly leaves, apple, peach, pear, tomato, cherry, apricot, banana, grape, loquat, longan peel, longan pulp, litchi peel, litchi pulp, soybean, peanut, corn, potato tuber, rose petals, pomegranate petals, shiitake mushroom, oyster mushroom and other specimens. The unique lysis buffer formula rapidly inactivates endogenous RNase and effectively eliminates interference caused by polysaccharides and polyphenols. No toxic reagents such as phenol or chloroform are required throughout the operation. RNA is purified via silica membrane adsorption. The obtained total RNA features high purity, free of genomic DNA, protein and other contaminants. It is well qualified for downstream experiments including Real-Time RT-PCR, RT-PCR, Northern Blot, Dot Blot, in vitro translation and other molecular biology applications.

Components

Components	50T
DNase I	2×1000 U
10×Reaction Buffer	1 mL
Buffer RLS	40 mL
Buffer RW1	40 mL
Buffer RW2*	11 mL
RNase-Free Water	10 mL
Spin Columns FS With Collection Tubes	50
Spin Columns RM With Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 mL)	50

Storage

DNase I and 10×Reaction Buffer at 2-8℃, all other components at room temperature (10-30℃).

Required Reagents and Equipment

- β-Mercaptoethanol
- Absolute ethanol (freshly opened or specially used for RNA extraction)

Instructions

Preparations

1. Precautions against RNase contamination
   - Use RNase-free plasticware and pipette tips.
   - Prepare solutions with RNase-free water.
   - Wear disposable masks and gloves, and change gloves frequently during operation.
   - Avoid repeated freeze-thaw cycles of samples to ensure RNA yield and integrity.
2. If precipitate appears in Buffer RLS, heat at 56℃ to dissolve completely, then cool to room temperature before use.
3. Add β-mercaptoethanol to Buffer RLS before use: add 20 μL β-mercaptoethanol per 1 mL Buffer RLS. The mixed solution can be stored at room temperature for one month.
4. Add absolute ethanol to Buffer RW2 as instructed on the label before first use.
5. All centrifugation steps are performed at 4℃ unless otherwise specified, and all operations should be finished quickly.
6. DNase I preparation: Add 500 μL RNase-free water into the DNase I vial, mix gently by inverting, do not vortex. The final concentration is 2 U/μL after full dissolution. Store at -20℃.

Protocol

1. Homogenization: Grind 50–100 mg plant tissue thoroughly into powder rapidly in liquid nitrogen. Add 500 μL Buffer RLS (verify β-mercaptoethanol has been added prior to use), then vortex vigorously immediately to mix well.
   Note: For high-moisture tissues such as watermelon pulp, tomato flesh and pear pulp, the sample weight can be increased appropriately up to a maximum of 200 mg. For starch-rich

samples or mature leaves, increase the volume of Buffer RLS properly, with a maximum volume of 700 µL.

2. Centrifuge at 12,000 rpm (~13,400×g) for 2 minutes at 4 °C.
3. Transfer the supernatant into Spin Columns FS placed in a collection tube. Centrifuge at 12,000 rpm for 1 minute at 4 °C. Carefully transfer the supernatant from the collection tube into a new self-prepared RNase-free centrifuge tube; avoid touching the cell debris pellet at the bottom with pipette tips.
4. Slowly add absolute ethanol equal to 0.5 times the supernatant volume and mix gently (precipitate may form). Load the mixture including precipitate into Spin Columns RM fitted in a collection tube. Split into two loading runs if the total volume exceeds column capacity. Centrifuge at 12,000 rpm for 1 minute at 4 °C, discard the flow-through and place the column back into the collection tube.
5. Add 350 µL Buffer RW1 into Spin Columns RM. Centrifuge at 12,000 rpm for 1 minute at 4 °C, discard waste liquid and reposition the column.
6. Preparation of DNase I Working Solution: Mix 52 µL RNase-free Water, 8 µL 10×Reaction Buffer and 20 µL DNase I (2 U/µL) evenly to prepare 80 µL total reaction mixture.
7. Directly add 80 µL prepared DNase I mixture onto the column membrane, incubate for 15 minutes at 20–30 °C.
8. Add 350 µL Buffer RW1 to Spin Columns RM, centrifuge at 12,000 rpm for 1 minute at 4 °C, discard flow-through and replace the column.
9. Add 500 µL Buffer RW2 (confirm absolute ethanol is added before use) into Spin Columns RM, centrifuge at 12,000 rpm for 1 minute at 4 °C, discard waste liquid and put the column back.
10. Repeat Step 9.
11. Centrifuge at 12,000 rpm for 2 minutes and discard flow-through.
    This step is to remove residual ethanol in the column, as residual ethanol will interfere with subsequent enzymatic reactions such as enzyme digestion and PCR.
12. Place Spin Columns RM into a new 1.5 mL RNase-free centrifuge tube. Drop 30–50 µL RNase-free Water vertically onto the center of the adsorption membrane without touching it. Stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm for 1 minute at 4 °C. Store the eluted RNA solution at -80 °C.

1) The volume of RNase-free Water shall not be less than 30 μL; insufficient volume will reduce RNA recovery rate.

2) For higher RNA yield, repeat elution with another 30–50 μL fresh RNase-free Water following Step 12.

3) For higher RNA concentration, reload the eluted solution back onto the column and repeat the centrifugation elution step in Step 12.