ARPENspin Plant total RNA Extraction Kit

Cat# ARP-PTRES50

Kit Components

COMPONENTS	
Lysis AG	25mL
Lysis BG	25mL
PLANTaid	2.5mL
PG Buffer	30mL
Wash Buffer	18mL (add 54mL ethanol before use)
RElution Buffer	10mL
MnCl2	450µL
DNase I	50µL
DNase Buffer	2mL
Spin Columns	50

Storage

• MnCl2, DNase I, and DNase Buffer are stored at - 20℃. Other components are stored at room temperature. The kit has demonstrated stability of 18 months.

• The kit can be transported at room temperature.

Introduction

The kit provides a ready-to-use reagent for isolating total RNA from plant tissues that contain polysaccharides and polyphenols. Start by adding the Lysis solution to the processed sample, and then transfer the mixture to the Spin column. Total RNA can be easily purified through several washing and elution steps. The isolated RNA can be widely used in various applications, including PCR, real-time PCR, sequencing, Southern blotting, mutant analysis, SNP analysis, and more.

The kit offers a straightforward, quick, and cost-effective method for isolating high-quality RNA, and it is suitable for high-throughput applications. The purified RNA can be used extensively in various applications, including Northern blotting, hybridization, poly(A)+ selection, in vitro translation, RNase protection assays, RT-PCR/real-time RT-PCR analysis, and the construction of cDNA libraries, among others.

Additional apparatus and materials required but not supplied

- Sterile 1.5mL microcentrifuge tubes
- 10μL/200μL/1000μL tips
- Microcentrifuge capable of 14,000g
- Absolute ethanol
- Vortex mixer
- β- mercaptoethanol
- Liquid nitrogen
- Mortar

Important Notes

1. Add the ethanol indicated on the bottle label to the wash buffer and mix well.cl.

When the room temperature is too low, Lysis AG and Lysis BG will crystallize and precipitate. Please dissolve them in a water bath at 50℃ before use..

Protocol

1. Lysis selection:

The kit contains two types of lysis buffers and a bottle of PLANTaid. For most plant samples, you can use Lysis AG for extraction. If you experience poor extraction results with Lysis AG, it is recommended to use Lysis BG (for example, Siraitia grosvenorii leaves) or Lysis BG with PLANTaid (for example, cotton wool fiber).

Reagent preparation

a. Lysis AG: Add β - mercaptoethanol to Lysis AG until the final concentration is 2%, i.e. adding 20 μL β - mercaptoethanol into 980 μL Lysis AG.

b. Lysis BG: Add β - mercaptoethanol to Lysis BG until the final concentration is 2%, i.e. adding 20 μL β - mercaptoethanol into 980 μL Lysis BG.

c. Lysis BG+ PLANTaid: Add β - mercaptoethanol to Lysis BG until the final concentration is 2%, and add PLANTaid to the mix until the final concentration is 9%, i.e. adding 20 μL β - mercaptoethanol and 100μL PLANTaid into 980 μL Lysis BG. Mix well.

PS: The Lysis is best used once; it can be stored at 4 ℃ for a month.

2. Sample pretreatment

a. To grind the plant tissue using liquid nitrogen, start by grinding the plant material into a fine powder. Weigh out 50-100 mg of the powder and place it into a 1.5 mL centrifuge tube. Add 500 µL of Lysis buffer or 550 µL of Lysis buffer (BG + PLANTaid) to the tube. Immediately shake the mixture vigorously until there are no visible lumps.

b. To grind plant tissue using liquid nitrogen, begin by reducing the plant material into a fine powder. Weigh out 50-100 mg of the powder and transfer it into a 1.5 mL centrifuge tube. Next, add either 500 µL of lysis buffer or 550 µL of lysis buffer (BG + PLANTaid) to the tube. Finally, shake the mixture vigorously until there are no visible lumps.

3. The lysate should be centrifuged for 5 minutes at 12,000 rpm. Carefully transfer all of the lysate supernatant into a new 1.5 mL centrifuge tube.

Note: If you want to obtain more RNA, you can try to collect additional lysate supernatant, but avoid sucking it.

4. For more accurate estimates of the lysate supernatant volume, add an amount of absolute ethanol equal to 0.5 times the volume of the lysate. Although this may cause precipitation, it does not affect the extraction process. Please mix immediately, but do not centrifuge.

5. Transfer the entire mixture into a spin column and centrifuge for 1 minute at 12,000 rpm. Discard the flow-through.

Note: Before proceeding to the next step, ensure that the mixture has passed through the spin column.

6. Discard the liquid in the outer tube and add 500 µL of Wash Buffer to the spin column. Please ensure that absolute ethanol has been added first. Centrifuge at 12,000 rpm for 1 minute, then discard the liquid in the collection tube.

7. To prepare the DNase I working solution, combine 40 µL of DNase Buffer, 9 µL of $MnCl_2$, and 1 µL of DNase I in a new RNase-free 1.5 mL centrifuge tube. Mix well. Add 50 µL of the DNase I working solution to the center of the spin column and incubate at room temperature for 15 minutes.

8. Add 600 µL of PG Buffer to the spin column and let it stand at room temperature for 30 seconds. Then, centrifuge for 30 seconds at 12,000 rpm and discard the flow-through.

9. Add 500 µL of Wash Buffer to the spin column and centrifuge for 30 seconds at 12,000 rpm. Discard the flow-through. Next, add 250 µL of Wash Buffer to the spin column and repeat the previous step.

10. Centrifuge for an additional 1 minute at 12,000 rpm. Carefully remove the Wash Buffer to prevent residual ethanol from inhibiting downstream reactions.
11. If you need to increase the RNA concentration, reload the elute into the center of the Spin column. Incubate at room temperature for 2 minutes, and then centrifuge for 1 minute at 12,000 rpm. The buffer in the centrifuge tube will contain the total RNA.

Troubleshooting:

1. When the room temperature is too low, Lysis AG and Lysis BG may precipitate and form crystals. Please dissolve them in a water bath at 50°C before use.
2. For first-time use, add absolute ethanol to the Wash Buffer, cap the bottle, and mix thoroughly.
3. Please wear gloves and clean all tips and Eppendorf tubes with DEPC-treated ddH2O to avoid RNase contamination. If possible, work in an ultra-clean cabinet.
4. If there is residual liquid left in the tube after transferring the supernatant to the spin column and centrifuging for 1 minute, this may indicate excessive sample volume or incomplete lysis, which can block the membrane. To resolve this, follow these steps:

a. Reduce the sample volume.

b. If a blockage occurs, use a tip to gently break the surface of the membrane and then centrifuge again. It is generally recommended to discard the sample if issues persist.

5. Ensure that you add 50 µL to 100 µL of elution buffer directly onto the center of the membrane. If the volume of elution buffer is less than 50 µL, it may not fully saturate the membrane.
6. Total RNA should be stored at 4°C or in an ice bath for immediate use in downstream experiments, such as RT-PCR. Alternatively, it can be stored at -80°C. Long-term storage should be avoided as RNA is highly susceptible to degradation, regardless of preservation methods. For optimal purity, it is recommended to store RNA samples in nitrogen or a suitable stock buffer.NA.