AFTSpin Complex Plant Fast RNA Extraction Kit

Cat. No. : RK30122

Product components

Components	Component number	Size 50 RXN	Storage
Buffer RL1	RM30140	25 mL	RT
Buffer RL2	RM30145	50 mL	RT
Buffer PR2	RM30141	35 mL	RT
Buffer WB2	RM30144	12 mL	RT
RNase-free Adsorption Column and Collection Tubes	RM30185	50 pcs	RT
gDNA Remove Column and Collection Tubes	RM30186	50 pcs	RT
Buffer AB	RM30146	5 mL	RT
1.5 mL RNase-free Centrifuge Tubes	RM30202	50 pcs	RT
RNase-free H_2O	RM30142	5 mL	RT

Product Description

This kit is used for RNA extraction from complex plants (polysaccharide & polyphenol-rich plants). The kit does not rely on toxic reagents such as phenol and chloroform, and can remove residual gDNA by silica gel column purification technology and quickly extract total plant RNA. Extracted RNA can be directly used in RT-PCR, qPCR and RNA library construction experiments.

Storage

This kit is guaranteed stable for 12 months when stored properly at room temperature. Low temperature storage is easy to cause sediment formation and affect the experimental results.

Precautions

1. This product is for scientific research use only by professionals and is not intended for clinical diagnosis or treatment.
2. Please wear a lab coat and disposable gloves for your safety and health.
3. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed greater than or equal to 13,000 rpm (~14,000 x g).
4. Buffer RL1, Buffer RL2 and Buffer PR2 contain irritating compounds. Please wear latex gloves during operation to avoid contamination of skin, eyes and clothing. If skin, eyes are contaminated, rinse with plenty of water or normal saline.
5. After each use, the reagent bottle should be tightly capped immediately to avoid volatilization, oxidation and pH change caused by long-term exposure to the air.
6. The kit removes the vast majority of DNA contamination in the system, and purified RNA can usually be used for downstream experimental operations without DNase I treatment. If downstream experiments are sensitive to trace DNA, DNase I can be used to further remove DNA contamination.
7. Please wear lab coat, disposable latex gloves, disposable mask, and use RNase-free consumables to avoid RNase contamination.

Operation Instruction

Preparation before the experiment

1. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer WB2 and mix thoroughly. Mark the reagent bottle to indicate that ethanol has already been added.
2. Check Buffer RL1 for precipitate. If there is precipitate, put the tube in a water bath at 65℃ until the precipitate disappears.
3. Before the experiment, in each tube, 50 μL Buffer AB can be added to 500 μL Buffer RL2. For multiple RNA extractions, a mixture can be prepared proportionally, and then put into 65℃ water bath to preheat.

User Protocol (please read the precautions first)

1. Sample processing: Take fresh or dry plant tissue, add liquid nitrogen, and thoroughly grind it into powder.

   Note: If the next step is not carried out immediately after grinding, please store sample powder at -80℃.

2. Transfer 500 μL **Buffer RL2*** to 1.5 mL RNase-free centrifuge tube, and add 50 μL **Buffer AB**. Mixing upside down, preheat the reagent mix in a water bath at 65℃. If there are multiple samples, a mixture can be prepared proportionally.

   Note: If Buffer RL2 precipitates, put the tube in a water bath at 65℃ until the precipitate disappears.

3. Weigh 100-150 mg* sample after grinding with liquid nitrogen, and transfer it into the centrifuge tube been preheated at 65 ℃ (Described in Step.2). Vortex oscillation for 30-60 sec to fully lyse the sample, thereby decreasing viscosity and increasing yield.

   Note: If the sample has large amount of moisture, sample amount can be increased appropriately.

4. Return the lysate to the water bath at 65 ℃ for 5 min, occasionally reverse the tube 1-2 times to facilitate digestion, centrifuge the lysate at 13,000 rpm (14,000 x g) for 10 min.
5. Collect the supernatant into a 1.5mL RNase-free centrifuge tube, add 0.5 times the volume of absolute ethanol, and immediately pipette up and down to mix well.
6. Transfer the supernatnant mixture to the gDNA Remove Column placed in the Collection Tube (If supernatant is too much, add it to the gDNA Remove Column with several times), centrifuge at 13,000 rpm (14,000 x g) for 2 min, and discard the filtrate.
7. Place the gDNA Remove Column in a clean 1.5mL RNase-free centrifuge tube, add 500 μL of **Buffer RL1**, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and collect the filtrate (RNA in filtrate).
8. Add 0.5 times the volume of absolute ethanol to the filtrate (precipitation may occur at this time, but it does not affect the extraction process), mix immediately and do not centrifuge.
9. Add the mixture to the RNase-free Adsorption Column placed in the Collection Tube, centrifuge at 13,000 rpm (14,000 x g) for 2 min, and discard the filtrate.
10. Place back the RNase-free Adsorption Column into the Collection Tube, add 700 μL of **Buffer PR2** to the Adsorption Column, incubated for 1 min at room temperature. Then centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.
11. Place back the RNase-free Adsorption Column into the collection tube, add 500 μL of **Buffer WB2** (**confirm adding 48 mL of absolute ethanol prior to the first use**) to the Adsorption Column, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.
12. Repeat Step 11 once.
13. Place back the RNase-free Adsorption Column into the Collection Tube, centrifuge the tube at 13,000 rpm (~14,000 x g) for 2 min to remove the remaining **Bufferd WB2** in the Adsorption Column.
14. Remove the RNase-free Adsorption Column and put it into a 1.5mL RNase-free centrifuge tube. Add 30-100 μL of RNase-free H_2O to the middle of the Adsorption Column and allow to stand at room temperature for 2 min. Centrifuge at 13,000 rpm (14,000 x g) for 1 min to elute the RNA.
15. The extracted RNA can be directly used for downstream experiments or stored at -80℃.