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
Buffer AB	RM30146	5 mL	RT
RNase-free H_2O	RM30142	5 mL	RT
RNase-free Adsorption Column and Collection Tubes	RM30185	50 pk	RT
gDNA Remove Column and Collection Tubes	RM30186	50 pk	RT
1.5 mL RNase-free Centrifuge Tubes	RM30202	50 pk	RT

Product Description

This kit is used for RNA extraction from common plants and complex plants (plants rich in polysaccharides and polyphenols). It doesn't rely on toxic reagents such as phenol and chloroform. By using silica column purification technology, it can remove residual gDNA and rapidly extract total plant RNA. The extracted RNA can be directly used in experiments such as RT-PCR, qPCR, and RNA library construction.

Storage

Store at room temperature (10-30℃).

Precautions

1. This product is for scientific research use only by professionals and is not intended for clinical diagnosis or treatment.
2. 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).
3. 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.
4. 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.
5. 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.
6. 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. Materials to Prepare: absolute ethanol, 1.5 mL RNase-free centrifuge tubes.
2. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer WB2 and mix thoroughly.
3. Check Buffer RL1 for precipitate. If there is precipitate, put the tube in a water bath at 65℃ until the precipitate disappears.
4. If extracting RNA from complex plants, 50 μL of Buffer AB can be added to each tube containing 500 μL of Buffer RL2 before the experiment. For the extraction of multiple samples, a mixture can be prepared proportionally, and then put into 65℃ water bath to preheat.

RNA Extraction from Common Plants

1. Sample Processing (Liquid Nitrogen Grinding is Recommended): Grind the plant samples into powder rapidly in liquid nitrogen. If the samples after liquid nitrogen grinding will not be subjected to the next operation immediately, please store them at -80°C.
2. Transfer 500 μL of **Buffer RL1** into a 1.5 mL RNase-free centrifuge tube (provided by the user), add 50 μL of **Buffer AB**, and mix well for later use. Weigh an appropriate amount (50-100 mg) of the sample ground in liquid nitrogen and add it to the above-mentioned 1.5 mL RNase-free centrifuge tube. Vortex for 30-60 seconds to ensure thorough lysis. Then centrifuge the lysate at 13,000 rpm (~14,000 x g) for 5 minutes.
3. Transfer the supernatant to a gDNA Removal Column placed in a collection tube. If there is a large amount of supernatant, add it to the gDNA Removal Column in multiple portions. Centrifuge at 13,000 rpm (~14,000 x g) for 2 minutes and **collect the filtrate**.
4. Add 0.5 times the volume of anhydrous ethanol to the filtrate and immediately pipette up and down to mix well.
5. Transfer the mixture from step 4 to an RNase-free Adsorption Column placed in a collection tube. Centrifuge at 13,000 rpm (~14,000 x g) for 2 minutes and **discard the filtrate**.
6. Put the RNase-free Adsorption Column back into the collection tube. Add 700 μL of **Buffer PR2** to the adsorption column. Centrifuge at 13,000 rpm (~14,000 x g) for 30 seconds and **discard the filtrate**.
7. Put the RNase-free adsorption column back into the collection tube. Add 500 μL of **Buffer WB2** (make sure 48 mL of absolute ethanol has been added before the first use) to the adsorption column. Centrifuge at 13,000 rpm (~14,000 x g) for 30 seconds and **discard the filtrate**.
8. Repeat step 7 once.
9. Put the RNase-free Adsorption Column back into the collection tube. Centrifuge the empty column at 13,000 rpm (~14,000 x g) for 2 minutes to remove the residual Buffer WB2 in the adsorption column.
10. Take out the RNase-free Adsorption Column and place it in a 1.5 mL RNase-free centrifuge tube. Add 30-100 μL of RNase-free H_2O to the center of the adsorption column. Let it stand at room temperature for 2 minutes, then centrifuge at 13,000 rpm (14,000 x g) for 1 minute to elute the RNA.
11. The extracted RNA can be used directly for downstream experiments or stored at -80°C.

RNA Extraction from Complex Plants

1. Sample Processing (Liquid Nitrogen Grinding is Recommended): Grind the plant samples into powder rapidly in liquid nitrogen. If the samples after liquid nitrogen grinding will not be subjected to the next operation immediately, please store them at -80°C.
2. Transfer 500 μL of **Buffer RL2*** into a 1.5 mL RNase-free centrifuge tube (provided by the user).Add 50 μL of **Buffer AB**, invert the tube to mix well, and then pre-heat it in a 65°C water bath. For multiple samples, prepare the reagents in proportion.

   Note: If there is precipitation in Buffer RL2, place it in a 65°C water bath to redissolve first.
3. Weigh an appropriate amount of the sample ground in liquid nitrogen (100-150 mg. If the sample contains a high amount of moisture, the sample amount can be increased appropriately). Transfer it to the above-mentioned centrifuge tube pre-heated at 65°C. Vortex for 30-60 seconds to ensure thorough lysis, thereby decreasing viscosity and increasing yield.
4. Briefly place the lysate back in the 65°C water bath for 5 minutes, and invert the tube 1-2 times occasionally to facilitate lysis. Then centrifuge the lysate at 13,000 rpm (14,000 x g) for 10 minutes.
5. Collect the supernatant into a 1.5 mL 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 mixture to a gDNA Removal Column placed in a collection tube. (If there is a large amount of supernatant, add it to the gDNA Removal Column in multiple portions.) Centrifuge at 13,000 rpm (14,000 x g) for 2 minutes and **discard the filtrate**.
7. Place the gDNA Removal Column in a clean 1.5 mL RNase-free centrifuge tube. Add 500 µL of **Buffer RL1** and centrifuge at 13,000 rpm (14,000 x g) for 30 seconds. **Collect the filtrate (the RNA is in the 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.) Immediately pipette up and down to mix well without centrifugation.
9. Transfer the mixture to an RNase-free Adsorption Column placed in a collection tube. Centrifuge at 13,000 rpm (14,000 x g) for 2 minutes and **discard the filtrate**.
10. Put the RNase-free Adsorption Column back into the collection tube. Add 700 µL of **Buffer PR2** to the adsorption column, let it stand at room temperature for 1 minute, then centrifuge at 13,000 rpm (14,000 x g) for 30 seconds and **discard the filtrate**.
11. Put the RNase-free Adsorption Column back into the collection tube. Add 500 µL of **Buffer WB2** (make sure 48 mL of absolute ethanol has been added before the first use). Centrifuge at 13,000 rpm (14,000 x g) for 30 seconds and **discard the filtrate**.
12. Repeat step 11 once.
13. Put the RNase-free Adsorption Column back into the collection tube. Centrifuge the empty column at 13,000 rpm (14,000 x g) for 2 minutes to remove the residual Buffer WB2 in the adsorption column.
14. Take out the RNase-free Adsorption Column and place it in a 1.5 mL RNase-free centrifuge tube. Add 30-100 µL of RNase-free H_2O to the middle of the adsorption column. Let it stand at room temperature for 2 minutes, then centrifuge at 13,000 rpm (14,000 x g) for 1 minute to elute the RNA.
15. The extracted RNA can be directly used for downstream experiments or stored at -80℃.