AFTSpin Universal Fast Plant RNA Extraction Kit

Cat. No. : RK30121

Product components

Components	Component number	Size 50 RXN	Storage
Buffer RL1	RM30140	25 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

The kit rapidly extracts RNA from simple plant cells as well as complex plant tissues, such as those with abundant polysaccharide polyphenols, using silica gel column purification technology that avoids the use of toxic reagents such as phenol and chloroform. Residual gDNA is removed using gDNA removal columns. Extracted RNA can be directly used for RT-PCR, qPCR, RNA library construction, and other experiments.

Storage

Reagents are stable for 12 months at room temperature. Storage at low temperatures should be avoided due to precipitation of reagents which can negatively affect performance.

Highlights

1. Avoids toxic reagents such as phenol and chloroform as well as ethanol precipitation.
2. Extraction of a single sample can generally be completed within 15 minutes.
3. Use of gDNA Removal Columns eliminates the need for DNase digestion of product RNA.
4. Produces reliable results from both simple plant and complex plant samples.

Scope of Application

This product enables rapid extraction of total RNA from plant tissue and cells, facilitates removal of gDNA residues, and generates quality RNA for use in RT-qPCR, RT-PCR, and a variety of other applications.

Operational Instructions

Materials to be prepared by the user

1. Absolute ethanol
2. 1.5 mL RNase-free centrifuge tubes

Reagent Preparation

1. WB2 buffer: Add **48 mL** of absolute ethanol to Buffer WB2 bottle prior to initial use.
2. Before use, Buffer RL1 and Buffer RL2 should be checked for precipitation; if observed, dissolve the precipitate in a 65℃ water bath. The solution can be used only after it becomes clear.
3. To extract RNA from complex plants, 50 μL of reducing agent Buffer AB can be added to each 500 μL tube of Buffer RL2, then mix and incubate in a 65℃ water bath. Prepare for extraction of multiple samples by scaling up reagent preparation proportionally as needed.

Precautions

1. This product is for scientific research use by professionals only and is not intended for clinical diagnosis or treatment.
2. Please wear a lab coat and disposable gloves for your safety and health.
3. All centrifugation steps should be performed at room temperature using a centrifuge at ≥ 13,000 rpm (~14,000 x g).
4. Buffer RL1, and Buffer PR2 contain irritating compounds. Please wear latex gloves while handling to avoid contamination of skin, eyes and clothes. If skin or eyes are contaminated, rinse with plenty of water or saline.
5. Reagent bottles should be tightly capped after each use to avoid volatilization, oxidation, and pH changes caused by prolonged exposure to the air.
6. This kit removes the vast majority of contaminating DNA in the purified RNA sample; the RNA can be used for experimental downstream operations without DNase I treatment. Should downstream experiments be very sensitive to DNA, DNase I may be used to further remove DNA contamination.
7. When using this kit, please wear lab coat, disposable latex gloves, disposable mask, and use RNase-free supplies to avoid RNase contamination.

User Protocol

1. Cell disruption:Cryogenic grinding is recommended for cell disruption. Process plant samples into fine powder using liquid nitrogen, and immediately store ground samples at -80℃ unless reserved for immediate use.
2. Transfer 500 μL of Buffer RL1 into a 1.5 mL RNase-free centrifuge tube, add 50 μL of Buffer AB, and mix well for later use. Weigh an appropriate amount of sample ground in liquid nitrogen (50-100 mg) and add to the above 1.5 mL Rnase-free centrifuge tube; vortex for 30-60 sec to fully lyse, and centrifuge the lysate at 13,000 rpm for 5 min.
3. Transfer the supernatant to a gDNA Removal Column with a collection tube in place (if supernatant volume is too large to loaded, the sample can be divided and added to the gDNA Removal Column in several portions) and centrifuged at 13,000 rpm for 2 minutes. **Retain 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. Add the mixture from step 4 to a RNase-free adsorption column with a collection tube in place, centrifuge at 13,000 rpm for 2 minutes, and **discard the filtrate.**
6. Return the RNase-free adsorption column to the collection tube and add 700 μL of Buffer PR2 to the adsorption column. Centrifuge at 13,000 rpm for 30 sec, and **discard the filtrate**.
7. Return RNase-free adsorption column to collection tube, add 500 μL of Buffer WB2 to the adsorption column (**Confirm the addition of absolute ethanol before use**). Centrifuge at 13,000 rpm for 30 sec, and **discard the filtrate**.
8. Repeat step 7 above once.
9. Place the RNase-free adsorption column back into the collection tube and centrifuge the empty tube at 13,000 rpm for 2 minutes to remove residual Buffer WB2 from the adsorption column (alternatively, the empty tube can be placed in a fume hood for 5 minutes until ethanol is completely evaporated).
10. Transfer the RNase-free adsorption column to a new RNase-free collection tube, add 30-100 μL of RNase-free dd H_2O to the center of the adsorption column, and allow to stand at room temperature for 2 minutes. Centrifuge at 13,000 rpm for 1 minute to elute the RNA.
11. The extracted RNA can be used directly for downstream experiments or stored at -80℃.