RNApure Plant Kit
Plant RNA Extraction Kit
Item No. R665489 (50 preps)
Storage conditions: room temperature (15-30° C)

Product content

individual parts making up a compound	R665489 50 preps
Buffer RL	35 ml
Buffer RLC	35 ml
Buffer RW1	40 ml
Buffer RW2 (concentrate)	11 ml
RNase-Free Water	10 ml
Spin Columns FL with Collection Tubes	50
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Product Introduction

This kit is used to extract and purify high quality total RNA from various plants, and is also suitable for the extraction of fungal mycelial RNA.
Unique Shredder separation columns for homogenization and filtration of highly viscous plant or fungal lysates while using silicone-based
Plasma membrane adsorption of RNA for purification allows various contaminants such as polysaccharides to be effectively removed by washing, and the eluted RNA can be Directly used in various downstream experiments. The molecular weight of RNA extracted from this kit is greater than 200 bases, with high purity and almost no DNA residues. In the case of RNA experiments that are very sensitive to trace DNA, residual DNA can be removed by digestion on a column using RNase-free DNase I. The extracted RNA can be used for Northern Blot, Dot Blot, RT-PCR and in vitro translation experiments.

Self-contained reagents: β-mercaptoethanol, anhydrous ethanol (freshly opened or for RNA extraction).

Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.

(2) Glassware should be dry baked at 180° C for 4 hours before use, and plasticware can be soaked in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. Avoid repeated freezing and thawing of the extracted samples, as this will affect the quantity and quality of the RNA extracted.
3. Add β-mercaptoethanol to Buffer RL before use to a final concentration of 1%. For example, add 10 μl of β-mercaptoethanol to 1 ml of Buffer RL. Buffer RL with β-mercaptoethanol can be stored at room temperature for 1 month. β-mercaptoethanol is not required for use of Buffer RLC.
4. Anhydrous ethanol should be added to Buffer RW2 according to the instructions on the label of the reagent bottle before first use.
5. If Buffer RL and Buffer RLC produce a precipitate, heat to dissolve and leave at room temperature.
6. All centrifugation steps are carried out at room temperature unless otherwise indicated and all steps are performed quickly.
7. If the downstream experiments are very sensitive to DNA, it is recommended that RNA be treated with RNase-free DNase I (Art. No. R665489).

Operation process

1. **Take 50-100 mg of fresh plant tissue**, add liquid nitrogen and quickly grind into powder.
2. Collect the ground powder into a centrifuge tube (supplied), add **600 μl of Buffer RL (check that β-mercaptoethanol has been added before use) or Buffer RLC**, and vortex and oscillate to fully lysate the powder.
Note: 1) The main component of Buffer RL is guanidine isothiocyanate, which is suitable for lysis of most plant tissues. However, in some plant tissues (such as endosperm of corn), due to the special secondary metabolites, guanidine isothiocyanate causes precipitation of the sample, resulting in poor RNA extraction, in this case, Buffer RLC can be added instead of Buffer RL.
2) Incubation at 56° C for 1-3 minutes helps tissue lysis, but do not incubate at high temperatures for plants with high starch content.
3. Transfer all of the liquid from step 2 to the filter columns (Spin Columns FL) in the collection tube at 12,000 rpm.
(~13,400 × g) centrifuge for 2 min and transfer the supernatant from the collection tube to a new centrifuge tube (self-contained).
Note: 1) The tip of the tip of the gun can be cut off when aspirating liquids to facilitate sampling.

(2) Spin Columns FL removes most of the debris, but a small portion will still flow out and form a precipitate in the collection tube after centrifugation, so be careful to avoid aspirating the precipitate when proceeding to the next step.

4. Add 0.5 times the volume of anhydrous ethanol to the clean lysate obtained in step 3 and mix rapidly.

Note: Precipitation may occur upon addition of ethanol, but does not affect subsequent tests.

5. Add all of the solution obtained in step 4 to the Spin Columns RM in the collection tube. If you cannot add all of the solution to the column at one time, transfer it in two passes. centrifuge the column at 12,000 rpm for 15 seconds, pour off the waste solution from the collection tube, and place the column back into the collection tube.

6. Add 700 μl of Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min and pour off the waste liquid in the collection tube.

Place the adsorption column back into the collection tube.

OPTIONAL STEP: If RNA experiments that are very sensitive to trace DNA are to be performed, replace step 6 with the following step.

1) Add 350 μl Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 15 seconds, discard the waste liquid, and re-adsorb the column.

Put it back into the collection tube.

2) Preparation of DNase I mixture: Take 52 μl of RNase-Free Water and add 8 μl of 10×Reaction to it.

Buffer and 20 μl DNase I (1 U/μl), mixed well, and prepared into a final volume of 80 μl of reaction solution.

Note: The above system is configured according to our product DNase I (CW2090S), please refer to the corresponding manual for other products.

3) Add 80 μl of DNase I reaction solution directly to the adsorption column and incubate at 20-30° C for 15 minutes.

4) Add 350 μl Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 15 seconds, discard the waste liquid, and re-adsorb the column.

Put it back into the collection tube.

7. Add 500 μl of Buffer RW2 to the column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 15 seconds, pour off the waste liquid in the collection tube, and put the column back into the collection tube.

8. Repeat step 7.

9. Centrifuge the column at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for a few minutes to dry thoroughly.

Anhydrous ethanol in dry adsorption column.

Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (zymography, PCR, etc.).

10. Place the adsorption column in a new RNase-free centrifuge tube and add 30-50 μl to the center of the column.

RNase-Free Water, leave at room temperature for 1 min, centrifuge at 12,000 rpm for 1 min, collect RNA solution, -70° C
Preserves RNA from degradation.
Note: 1) The volume of RNase-Free Water should not be less than 30 μl, too small a volume affects the recovery.
2) If you want to increase the RNA yield, repeat step 10 with 30-50 μl of fresh RNase-Free Water.
3) If you want to increase the concentration of RNA, reintroduce the obtained solution into the adsorption column and repeat step 10.