Plant RNA Extraction Kit (DNase I)

Item No. R669988

Storage conditions: -20° C.

Products

individual parts making up a compound	50T
DNase I	1000U
10×Reaction Buffer	1000μl
Buffer RL	35ml
Buffer RLC	35ml
Buffer RW1	40ml
Buffer RW2 (concentrate)	11ml
RNase-Free Water	10ml
Spin Columns FL with Collection Tubes	50
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Products

This kit is used for the extraction and purification of high-quality total RNA from a variety of plants, and is also suitable for the extraction of fungal mycelial RNA. The unique separation column is used for homogenization and filtration of high viscosity plant or fungal lysates, while the silicon matrix membrane is used to adsorb the RNA for purification, so that various contaminants, such as polysaccharides, are effectively removed by washing, and the eluted RNA can be directly used in various downstream experiments. The molecular weight of RNA extracted by this kit is more than 200 bases, with high purity and almost no DNA residue. For RNA experiments that are very sensitive to trace DNA, the residual DNA can be removed by digestion on a column using RNase-free DNase. The extracted RNA can be used in 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) RNase-free water should be used to prepare the solution.
(3) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. 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-roasted at 180° C for 4 hours before use, and plasticware can be soaked in 0.5M 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.
3. Avoid repeated freezing and thawing of the extracted samples, otherwise it will affect the amount and quality of RNA extraction.
4. Please add β-mercaptoethanol to Buffer RL before use, add 10μl of β-mercaptoethanol to 1ml of Buffer RL, it can be stored for 1 month at room temperature. Buffer RL with β-mercaptoethanol can be stored at room temperature for 1 month. β-mercaptoethanol is not required for use of Buffer RLC.
5. Anhydrous ethanol should be added to Buffer RW2 before first use according to the instructions on the reagent bottle label.
6. If precipitation occurs in Buffer RL and Buffer RLC, heat to dissolve and leave at room temperature.
7. All centrifugation steps are carried out at room temperature and all steps are performed quickly.

Procedure

1. 50-100 mg of plant tissue is quickly ground to a powder in liquid nitrogen and added to 600 μl of Buffer RL (check for addition of β-mercaptoethanol before use) or Buffer RLC. vortexing and oscillating to allow for adequate lysis.
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 (e.g. 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.
2. Transfer all the liquid obtained in step 1 to an adsorption column (Spin Columns FL) that has been loaded into a collection tube, centrifuge at 12,000 rpm (~13,400

x g) for 2 minutes, and transfer the supernatant from the collection tube to a new centrifuge tube (supplied).

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 a precipitate will form in the collection tube after centrifugation, so be careful to avoid aspirating the precipitate when proceeding to the next step.

3. Add 0.5 times the volume of anhydrous ethanol to the clean lysate obtained in step 2 and mix rapidly.

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

4. Transfer the solution obtained in the previous step to the Spin Columns RM in the collection tube. If it is not possible to add all of the solution to the column at one time, centrifuge the column at 12,000 rpm for 15 seconds in two batches, discard the waste solution and put the column back into the collection tube.

5. Add 350 μl Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid and put the adsorbent column back into the collection tube.

6. Preparation of DNase I mixture: Take 52μl of RNase-Free Water, add 8μl of 10×Reaction Buffer and 20μl of DNase I (1U/μl) to it, mix well, and make a final volume of 80μl of reaction solution.

7. Add 80μl of DNase I mixture directly to the adsorption column and incubate at 20-30° C for 15 minutes.

8. Add 350 μl of Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 1 minute, discard the waste liquid and put the column back into the collection tube.

9. 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, and discard the waste solution.

10. Repeat step 9.

11. Place the adsorbent column back into the collection tube, centrifuge at 12,000 rpm for 1 minute, and allow the column to come to room temperature for a few minutes to thoroughly dry out the anhydrous ethanol in the adsorbent 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.).

12. Load the adsorption column into a new centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorbent membrane, leave it at room temperature for 1 minute, centrifuge at 12,000 rpm for 1 minute, and store the resulting RNA solution at -70° C to prevent degradation.

Note: 1) The volume of RNase-Free Water should not be less than 30 μl, too small volume affects the recovery rate.

2) If you want to increase the RNA yield, repeat step 12 with 30-50 μl of fresh RNase-Free Water.

3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 12 repeated.