For research use only Version Number：1.0-1312

Plant Total RNA Isolation Kit

For total RNA purification from general plant samples containing low polysaccharide and polyphenol components

Component	RE-05011	RE-05014
	50T	200T
Buffer PRL1*	25 ml	100 ml
Buffer PRL2	16.5 ml	66 ml
Buffer PRW1	25 ml	100 ml
Buffer PRW2	24 ml	96 ml
RNase-Free ddH_2O	10 ml	40 ml
RNA-Only Column	50	200
DNA-Cleaning Column	50	200
Manual	1	1

*: Buffer PSL1 and Buffer PRW1 contain irritating chaotropic salts. Please wear gloves and take relevant protective measures during operation.

Product Introduction

This kit uses the spin column and formula developed by our company, which can efficiently extract high-purity and high-quality total RNA from various plant tissues with low polysaccharides and polyphenols; if plant samples are polysaccharides and polyphenols, it is recommended to use Plant Total RNA Isolation Kit Plus for better RNA extraction results. Provide DNA-Cleaning Column to easily separate the supernatant and tissue lysate, remove the DNA in the sample, easy to operate and time-saving.

The whole system is RNase-Free, so that the extracted RNA is not degraded; Buffer PRW1, Buffer PRW2 buffer washing system makes the obtained RNA extremely pure.

Storage condition

This kit can be stored for 24 months under dry conditions at room temperature (15-25°C); if it needs to be stored for a longer period of time, it can be stored at 2-8°C. Buffer PRL1 can be placed at 4°C for 1 month after adding β-mercaptoethanol (optional) (it is recommended to add when you use it).

Note: If stored at low temperature, the solution is prone to precipitation. Before use, be sure to place the solution in the kit at room temperature for a period of time. If necessary, preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate, and mix it before use.

RNA yield and Quality

RNA can be purified from a variety of plant tissues with plant total RNA extraction kits. RNA yield varies with sample type, quality and the operation. The following is the yield and purity of 50 mg of RNA from various plant samples extracted using this kit. In actual operation, there may be discrepancies with this data.

Fresh tender leaves (50 mg)	RNA yield(μg)	OD260/280	OD260/230
Tobacco	27-30	1.8-2.1	1.8-2.1
Tomato	32-35	1.8-2.1	1.8-2.1
Soybeans	31-34	1.8-2.1	1.8-2.1
Corn	15-17	1.8-2.1	1.8-2.1
Rape	8-10	1.8-2.1	1.8-2.0
Rice	15-17	1.8-2.1	1.8-2.1

Note: The above data are obtained from fresh plant leaves. If the plant leaves are older, or other parts of the plant (such as leaf veins, rhizomes, etc.), or the sample has been stored for too long, the RNA yield may be lower than the data above.

Precautions:

- All experimental steps are performed at room temperature (15-25°C) (including centrifugation). Do not use ice bath and low-temperature (4°C) centrifugation.
- The sample should avoid repeated freezing and thawing, otherwise the extracted RNA will be degraded and the extraction amount will also decrease.
- The dosage of fresh plant leaves should not exceed 50 mg, otherwise it will affect the RNA yield and purity.
- Before using the kit, add β-mercaptoethanol to Buffer PRL1. Add 10 μl β-mercaptoethanol to every 500 μl Buffer PRL1. It is recommended to prepare it for immediate use. After adding β-mercaptoethanol, Buffer PRL1 can be stored at 4°C for 1 month. If the extracted RNA is not used for cloning full-length cDNA, but only for other downstream operations such as qPCR or sequencing analysis, you can choose not to add β-mercaptoethanol, which will not affect the extraction effect.
- Before using the kit, add absolute ethanol to Buffer PRL2. Please refer to the label on the reagent bottle for the amount added.
- Before using the kit, add absolute ethanol to Buffer PRW2. Please refer to the label on the reagent bottle for the amount added.
- RNA yield and quality are related to the amount of cell sample and elution volume. It is recommended to use 50 mg of tissue per 500μl Buffer PRL1.
- Elution volume: The elution volume should not be less than 50 μl, otherwise it will affect the RNA recovery efficiency.

◆ Please check whether there is crystal precipitation in Buffer PRL1 and Buffer PRW1 in the kit. If crystals are precipitated after storage at low temperature, place the Buffer at room temperature or 37°C for a period of time, dissolve the crystals and mix well before using.

Operation steps (full operation at room temperature (15-25°C), do not ice bath and low-temperature centrifugation)

Please add absolute ethanol to Buffer PRL2 and Buffer PRW2 before use. Please refer to the label on the bottle for the added volume.

1. Take 500 µl Buffer PRL1 into a 2 ml centrifuge tube, add 10 µl β-mercaptoethanol (you need to prepare yourself), mix well and set aside.
2. Take an appropriate amount of fresh plant leaves or tissues, cut them as much as possible, place them in a pre-cooled mortar, and add liquid nitrogen to fully grind them.
3. Quickly weigh 50 mg of ground fresh plant leaf powder, transfer to Buffer PRL1, shake vigorously to mix, and let it stand at room temperature for 5 min.

   Note: The amount of tissue should not exceed 50 mg, otherwise the quality of RNA will decrease. Transfer quickly before the plant leaf powder melts, and after the cells are broken, RNA is extremely prone to degradation in a non-freezing environment.
4. (Optional step) If you find that there are obvious tissue fragments in the solution after tissue lysis or the solution is too viscous, centrifuge at 12,000 rpm (~13,400 ×g) at room temperature for 2-5 minutes, take the supernatant and proceed to the next step; If the solution has no visible fragments or is clear, please ignore this step.
5. Transfer all the supernatant to the DNA-Cleaning Column (put the DNA-Cleaning Column into the collection tube), and centrifuge at 13,300 rpm (~17,000 × g) for 2 min. Remove the DNA-Cleaning Column and keep the supernatant in the collection tube.

   Note: The plant tissue lysate is relatively viscous, and the tip of the pipette tip can be cut off when transferring the liquid to facilitate sampling. Although most of the cell debris is trapped on the membrane of the DNA-Cleaning Column, a small amount of cell debris will pass through the DNA-Cleaning Column and exist as a precipitate at the bottom of the collection tube. Carefully transfer the supernatant to a clean centrifuge tube and proceed to step 6. Do not aspirate the precipitate into the supernatant.
6. Carefully transfer the supernatant filtered by the DNA-Cleaning Column to a new 2 ml RNase-Free centrifuge tube (you need to prepare your own), and add 1.7 times to it (the volume should be about 500 µl supernatant). About 850 µl) volume of Buffer PRL2 (make sure to add absolute ethanol according to the instructions before use), mix gently to prepare for the following RNA purification steps. If precipitation occurs, do not perform centrifugation.

   Note: The amount of Buffer PRL2 added should be converted according to the volume of the supernatant in the actual operation. For example: add 850 µl Buffer PRL2 to 500 µl supernatant (alcohol has been added). The mixed solution may appear turbid or flocculent, please proceed directly to step 7.
7. Carefully transfer 750 µl of the above mixture to the RNA-only Column (the RNA-only Column is placed in the collection tube), gently cover the spin column lid, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard Collect the waste liquid in the tube.

   Note: If flocculent precipitates appear in the mixed solution, please transfer the precipitates to the RNA-only Column.
8. Put the RNA-only Column back into the collection tube, add all the remaining mixture to the RNA-only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard the waste in the collection tube.
9. Add 500 µl Buffer PRW1 to the RNA-only Column, gently cover the spin column lid, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard the waste in the collection tube.

   Note: After the centrifugation is completed, carefully remove the RNA-only Column, and do not let the bottom of the spin column touch the waste liquid in the collection tube. Pay attention to this detail in steps 9, 10, and 11.
10. Add 700 µl of absolute ethanol to the RNA-only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard the waste liquid in the collection tube.
11. Add 700 µl Buffer PRW2 to the RNA-only Column (make sure to add absolute ethanol according to the instructions before use), centrifuge at 12,000 rpm (~13,400 ×g) for 1 min, and discard the waste in the collection tube..
12. Repeat step 11.
13. Put the RNA-only Column back into the collection tube, centrifuge the empty tube at 12,000 rpm (~13,400 ×g) for 2 minutes, and discard the collection tube.

    Note: Centrifuge the empty tube for a long time to ensure that the residual ethanol on the spin column is cleaned. The residual ethanol will affect the downstream experiment.
14. Transfer the RNA-only Column to a new centrifuge tube, and add 50-200 µl of RNase-Free ddH2O preheated at 65°C to the center of the RNA-only Column membrane (do not add the eluent to the pressure Circle it, otherwise it will lose a larger volume of eluent), and leave it at room temperature for 2 minutes. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min to collect the RNA solution.