Version Number：1.0

Plant Total RNA Isolation Kit Plus

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

Kit composition	RE-05021	RE-05024
	50 T	200 T
Buffer PSL1*	25 ml	100 ml
Buffer PS	6 ml	24 ml
Buffer PSL2	24 ml	96 ml
Buffer PRW1	25 ml	100 ml
Buffer PRW 2	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

The 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. Provide DNA-Cleaning Column to easily separate the supernatant from the tissue lysate and remove the DNA in the sample, which is simple and time-saving.

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

Storage condition

This kit can be stored for 24 months under dry conditions at room temperature (15-25℃) ; If it needs to be stored for a longer time, it can be stored at 2-8℃. Buffer PSL1 can be placed at 4℃ for 1 month after adding β-mercaptoethanol (optional).

RNA yield and Quality

The yield is related to the plant sample itself, the initial amount of the sample, the freshness of the sample, the preservation time of the sample, and the operation. The following is the RNA yield and purity data extracted using 50 mg plant samples. In actual operation, there may be discrepancies with the data.

Fresh leaves (50 mg)	RNA yield (μg)	OD260/280	OD260/230
Banana	18-20	1.8-2.1	1.8-2.1
Ginkgo	18-20	1.8-2.1	1.8-2.1
Cotton	12-15	1.8-2.1	1.8-2.1
Pomegranate	21-23	1.8-2.1	1.8-1.9

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 in the table above.

Precautions:

◆ All experimental steps were at room temperature (15-25 ℃) for (including centrifugation) , **never using an ice bath and a low temperature (4 ℃) centrifuge**.

◆The sample should avoid repeated freezing and thawing, otherwise the extracted RNA will be degraded and the extraction amount will also decrease.

◆ Fresh plant leaves in an amount not more than 50 mg, otherwise it will affect the RNA yield and purity.

◆ Reagent cassette used before, in the Buffer PSLl added to the β-mercaptoethanol. Add 10 μl β-mercaptoethanol to every 500 μl Buffer PSL1. **It is recommended to prepare it for immediate use**. After adding β-mercaptoethanol Buffer PSL1 can be stored at 4 ℃ 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, please add ethanol absolute to Buffer PSL2. Please refer to the label on the reagent bottle for the amount added.

◆Before using the kit, please add ethanol absolute to Buffer PRW2. Please refer to the label on the reagent bottle for the amount added.

◆ Elution volume: elution volume of not less than 50μl, otherwise it will affect RNA recovery efficiency.

◆ Please check whether there is crystal precipitation in Buffer PSL1 and Buffer PRW1 in the kit. If crystals are precipitated after storage at low temperature, place the Buffer at room temperature or 37 ℃ for a period of time, dissolve the crystals and mix well before using.

Operation steps (Full operation at room temperature (15-25℃), do not ice bath and low-temperature centrifugation)

Please add ethanol absolute to Buffer PSL2 and Buffer PRW2 before use. Please refer to the label on the bottle for the added volume.

1. Take 500 µl Buffer PSLI in 2ml centrifuge tube was added **10** µl β-mercaptoethanol (prepared by yourself), Mix well for use.
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 weighed **50 mg** freshly abraded plant foliage or tissue powder was transferred to Buffer PSLI vigorously vortexed, allowed to stand at room temperature 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. Add 100 µl Buffer PS to the above liquid and mix gently.
5. (Optional step) If 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 min, take the supernatant and proceed to the next step; if the solution No visible fragments, clarification, please ignore this step.
6. All the supernatant was transferred to DNA-Cleaning Column the (DNA-Cleaning Column into a collection tube), 13,300 RPM (~ 17,000 × g) was centrifuged 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 7. Do not aspirate the precipitate into the supernatant.
7. 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 1.5 times (the volume should be about 600 µl of the supernatant) to it (Approximately 900 µl) volume of Buffer PSL2 (make sure to add ethanol absolute according to the instructions before use), mix gently to prepare for the following RNA purification steps. If precipitation occurs, do not perform centrifugation.
8. 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. Discard the waste liquid in the collection tube.
   Note: If flocculent precipitate appears in the mixed solution, please transfer the precipitate to the RNA-only Column.
9. 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.
10. 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 liquid 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. Steps 10, 11, and 12 all need to pay attention to this detail.
11. Add 700 µl of ethanol 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.
12. Add 700 µl Buffer PRW2 to the RNA-only Column (make sure to add ethanol absolute 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.
13. Repeat steps 12.
14. 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.
15. Transfer the RNA-only Column to a new centrifuge tube, and add 50-200 µl of RNase-Free ddH_2O preheated at 65°C to the center of the RNA-only Column membrane (do not add the eluate 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.
    Note: The volume of RNase-Free ddH_2O added should not be less than 50 µl. If the volume is too small, it will affect the elution efficiency. To increase the RNA yield, add the RNA solution obtained by centrifugation to the RNA-only Column again, and repeat step14.

The resulting RNA solution can be used directly in downstream experiments or stored at -80 ℃ . As this kit protects RNA secondary structure well, it is recommended that the RNA solution be denatured at 72 ℃ for 5-10 min before gel electrophoresis .