RNA Extraction Kit for Polysaccharide/polyphenol plants (gDNA Removal Column)

Code No.：NG3021S 50T

Contents	storage	50 T
Buffer PLS	RT	50 ml
Buffer RB	RT	25 ml
Buffer RE	RT	40 ml
Buffer RW	RT	10 ml Add absolute alcohol before use
RNase-free H_2O	RT	5 ml
Spin Columns RA/ Collection tubes	RT	50
Spin Columns gDNA/ Collection tubes	RT	50

This product can be stored at room temperature for 12 months without affecting its effectiveness.

Important Note：

All solutions should be clear. If the ambient temperature is low, the solutions may form precipitates and should not be used directly. In such cases, they be heated in a 37 ℃ water bath for a few minutes to restore clarity. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure the reagents to air, the caps of each solution should be tightly closed immediately after use.

Introduction：

This product is a unique product independently developed by our company. Its principle is completely different from the isothiocyanate guanidine/phenol/chloroform extraction, and it can efficiently separate RNA from other plant polysaccharides, while also effectively removing polyphenols and other plant secondary metabolites. It is suitable for the vast majority of plant samples (roots stems, leaves, and fruits), and has been validated in nearly a hundred types of plants (including highly challenging conifers). This product can also be used for the total RNA extraction of fungal samples.

This product utilizes the latest genomic DNA removal column technology to ensure the effective removal of gDNA. The resulting RNA does not require DNase digestion and can directly used for experiments such as reverse transcription PCR and quantitative real-time PCR. A unique lysis buffer rapidly lyses cells and inactivates cellular RNases, after which the lysate is through a genomic DNA removal column, where genomic DNA is removed and RNA passes through the filter. After the filtered RNA is adjusted for binding conditions with ethanol, it is selectively adsorbed onto silica matrix membrane in the spin column under high-chaotropic salt conditions. Then, through a series of rapid wash-and-centrifugation steps, impurities such as cellular and proteins are removed. Finally, low-salt RNase-free water elutes the purified RNA from the silica matrix membrane.

Safety Information:

1. Lysis buffer PLS, RB and RE contain irritating and harmful compounds. Wear latex gloves during operation to avoid contact with skin, eyes and clothing. If contact with skin or eyes occurs, rinse with plenty of water or saline.
2. Simple and fast, single sample operation can generally be completed within 25 minutes, the simplest and fastest kit in the world.

3. Multiple rinsing steps to remove proteins result in higher RNA purity.
4. Good repeatability.
5. Need to provide your own 2-mercaptoethanol, ethanol, etc.
6. The sample loading amount must absolutely not exceed the processing capacity of the genomic DNA removal column and the RNA adsorption column RA, otherwise it will cause DNA or a decrease in yield. When starting to optimize experimental conditions, if the DNA/RNA content of the sample is unknown, a smaller sample loading amount can be used, and the amount can be increased or decreased in the future based on the sample test results.
7. In some special cases where excessive DNA content causes residues, treat the RNA extract with RNase-free DNase I to improve the effect.

Operation Procedure:

Tip: Before the first use, please add absolute alcohol in the buffer RW and 70% ethanol and mark it.

1. Transfer 1 ml of buffer PLS into a centrifuge tube (if PLS has precipitated or formed a sediment, it must be redissolved in a 65 ℃ water bath first), and add 5% 2-Mercaptoethanol to the lysis buffer PLS. Mix and preheat in a 65 ℃ water bath. For multiple samples, scale up the preparation proportionally. 2-Mercaptoethanol is a key component of lysis buffer PB, and its final concentration can be increased to 10-20% if necessary.
2. Grind fresh or -70 ℃ frozen materials into a fine powder in liquid nitrogen.
3. Transfer 100-200 mg of fine powder (for samples with low water content such as leaves and seeds, 100-150 mg can be added; for samples with high water content such as strawberry and watermelon fruits, more can be added) into a preheated 1 ml buffer PLS (1ml PLS plus 50 µl 2-Mercaptoethanol) centrifuge tube. Immediately vortex for 30-60 seconds or mix by pip up and down to lyse, then place back into a 65 ℃ water bath a short time (5-10 min), occasionally inverting 1-2 times in between to aid lysis.
4. Centrifuge at 12,000 rpm for 10 min at 4 ℃. Pipette the aqueous phase out into RNase-free tube (If there are floating materials on the surface of the supernatant, simply use the pipette tip to bypass them and aspirate the liquid underneath.).
5. Add 1/2 volume ethanol. Transfer the resulting solution and any possible precipitate into a to Spin Columns gDNA, 12,000 rpm for 1 min or more time until all the liquid has passed through the membrane, discard the filtrate.
6. Place the Spin Columns gDNA into a clean 2 ml centrifuge tube, add 500 µl of Buffer RB, centrifuge at 12,000 rpm for 30 seconds, and collect the filtrate (RNA is in the filtrate, usually around 500 µl,).
7. Add 1/2 volume of absolute ethanol, a precipitate may form at this time, but it does not affect the extraction process. Immediately pipette up and down to mix, do not centrifuge. Transfer the resulting solution and any possible precipitate into a adsorption column RA.
8. Centrifuge for 2 min at 12,000 rpm. Discard the flow-through and set the Spin Column RA back into the Collection Tube.
9. Add 700 µl of Buffer RE to the Spin Column and centrifuge for 1 min, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
10. Add 500 µl of Buffer RW to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
11. Repeat step 10.
12. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
13. Add 30-50 µl of RNase-free H_2O to the center of the membrane, let the column stand for 2 min, then centrifuge for 1 min at 12,000 rpm. Stored at -70 ℃.