Rapid RNA Extraction Kit for plant

Code No.： NG312S 50 T

NG312M 100 T

Contents	storage	50 T	100 T
Buffer PB	RT	50 ml	100 ml
Buffer RE	RT	25 ml	50 ml
Buffer RW	RT	10 ml	25 ml
		Add absolute alcohol before use	
70% ethanol	RT	9 ml	18 ml
		Add absolute alcohol before use	
RNase-free H_2O	RT	10 ml	20 ml
Spin Columns RA	RT	50	100
RNase-free filter column	RT	50	100
Collection tubes（2 ml）	RT	100	200

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. Improper storage at low temperatures (4 ℃ or -20 ℃) can precipitation in the solutions and affect the performance, therefore both transportation and storage should be carried out at room temperature (15 ℃ – 25 ℃). 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.

Safety Information:

1. All centrifugation steps were performed at room temperature unless otherwise specified.
2. Lysis buffer PB 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.
3. The unique PB lysis buffer formulation can effectively eliminate genomic contamination.
4. Multiple rinsing steps to remove proteins result in higher RNA purity.
5. Good repeatability.

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 lysis buffer PB into a centrifuge tube (if PB 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 PB. 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. For particularly complex plants, you can try adding PVP40 to the lysis buffer to a final concentration of 2%.
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 PB (1ml PB 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 filter column (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. Centrifuge at 12,000 rpm for 45 s at 4 ℃, Collect the filtrate (containing total RNA) to a new tube.
6. Add 1 volume of 70% ethanol (please check if anhydrous ethanol has been added first!), invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a micro-adsorption column RA (the adsorption column is placed inside a collection tube).
7. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column RA back into the Collection Tube.
8. Add 500 μl of Buffer RE 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.
9. Add 600 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.
10. Repeat step7.
11. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
12. Add 30-80 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 ℃.