ELK Biotechnology

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# Magbead Plant RNA Kit

| Catalog No. | Specification | Storage/Shelf life |
|---|---|---|
| EP025 | 96T | Room temperature/1 year |

## Product Introduction

This kit provides a simple and efficient automated plant RNA extraction solution. The RNA in the lysate binds to the surface of silica-coated magnetic beads in the presence of high salt, and is then washed with a rinse solution to remove impurities such as proteins. Then add DNase I removes DNA, and finally RNA is rinsed and eluted in RNase-Free Water. The total RNA obtained is of high purity, free of contamination from genomic DNA , proteins and other impurities, and can be used for Real Time RT - PCR , RT-PCR, Northern Blot、 Dot Blot and in vitro translation and other downstream experiments.

Storage conditions: DNase I and 10×Reaction Buffer Store at -20, other components at room temperature (15-30℃).

## Product Content

| Component | 96T |
|---|---|
| DNase I (1 U/μL) | 2×1 mL |
| 10×Reaction Buffer | 1 mL |
| Buffer PRL | 60 mL |
| Buffer PA | 40 mL |
| Buffer PGW1 (concentrate) | 25 mL |
| Buffer RW2 (concentrate) | 40 mL |
| RNase - Free Water | 10 mL |
| Magbeads PN | 2×1 mL |

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## Self-provided instruments and reagents

1. Centrifuge, 96 DW Plate , 8 tip Com、 Spin tips pack, RNase-free EP tube, RNase -free pipette tip, mortar, etc.
2. Ethanol (newly opened or for RNA extraction only).

## Preparation before the experiment and important precautions

1. Preparation before the experiment: For newly opened kits, follow the instructions on the reagent bottle label to prepare Buffer PGW1 (concentrate). and Buffer Add the corresponding amount of anhydrous ethanol to RW2 (concentrate ). Check whether anhydrous ethanol has been added before use. After adding anhydrous ethanol, the bottle cap must be tightened to prevent volatilization.
2. Note:
2.1 Operators should wear disposable masks and gloves, change gloves frequently during the experiment, and use RNase -free plastic products and Tips to avoid RNase contamination.
2.2 Plant tissues with high starch content (such as dry wheat seeds, dry corn seeds, etc.) will react with the lysis solution to produce a gelatinous substance. and The longer the lysis time, the more colloidal substances there are. We do not recommend using this kit for extraction. Please contact us to request another lysis buffer.
2.3 It is recommended to use fresh plant samples for extraction. If extraction is not possible in time, the samples should be frozen in liquid nitrogen and stored below -70 ° C. At the same time, repeated freezing and thawing should be avoided, otherwise the RNA extraction rate and quality will be affected.
2.4 Preparation of DNase I Mixed liquid: Take 52μL RNase-Free Water, add 8μL 10× Reaction Buffer and 20μL DNase I (1U/μL ), mix well and prepare a final volume of 80 μL of reaction solution.

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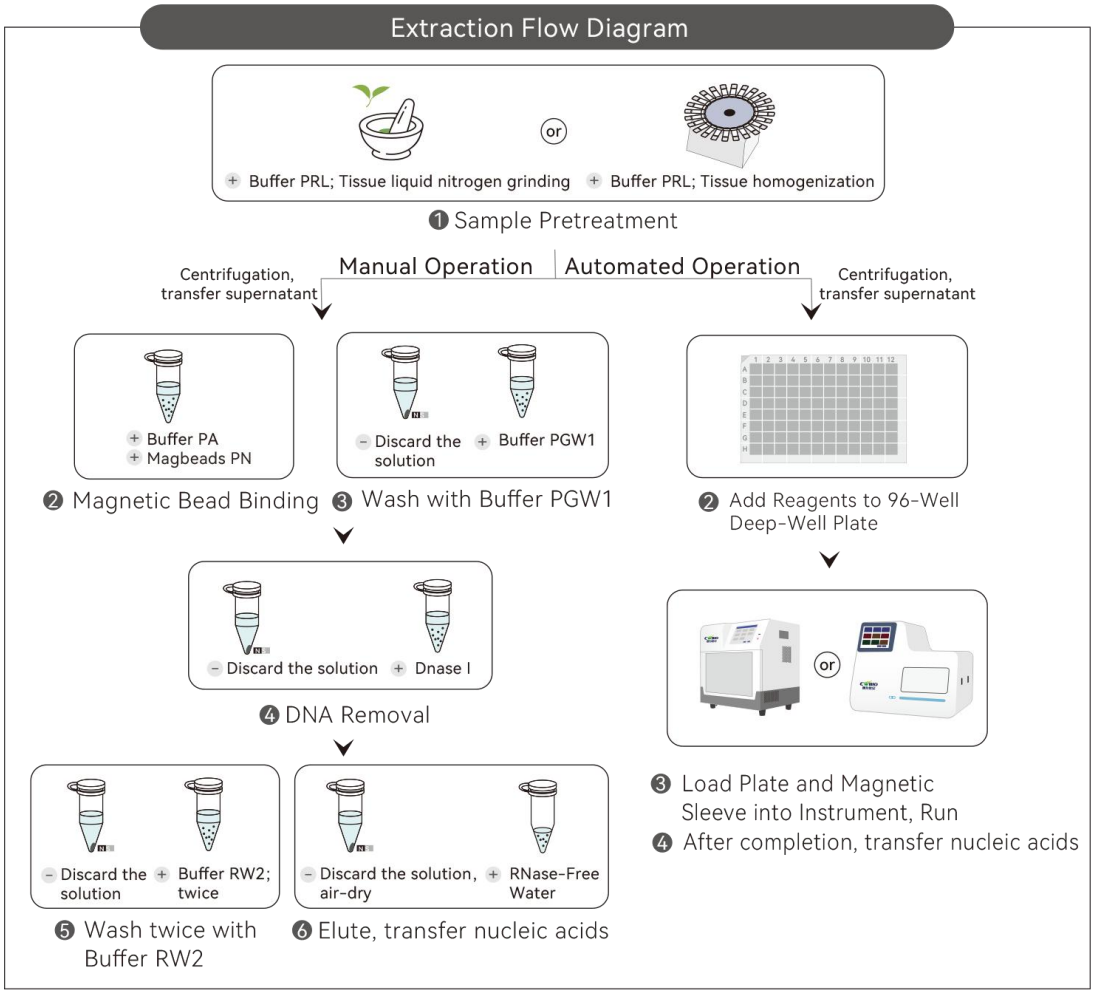
## Procedure




## Sample pretreatment

1. Disintegration of plant tissue:

1) Solution 1: Take 30-100 mg of fresh plant tissue or frozen plant tissue and quickly grind it into powder in liquid nitrogen. 600μL Buffer PRL was immediately vortexed to mix thoroughly.

2) Solution 2: Towards 2.0 Add 30-100 mL into the centrifuge tube mg fresh plant tissue or frozen plant tissue, 600μL Buffer PRL and 1 steel ball, and then immediately fix the centrifuge tube in a tissue disruptor and shake it;

2. 4℃,14,000 Centrifuge at 5 rpm After 5 min, transfer all the supernatant (about 500 μL) to a new 1.5 mL centrifuge tube (self-prepared).

Note: When aspirating the supernatant, try to avoid aspirating suspended matter. A small amount of suspended matter is normal. If there is a lot of suspended matter, the centrifugation time can be extended.

## Manual operation

1. (Continued from sample preparation) Go to 1.5 Add 350 mL into the centrifuge tube μL Buffer PA and 20 μL Magbeads PN, vortexed and incubated on a thermomixer at room temperature at 1700 rpm shake mixing 5 min. After instant centrifugation, place on a magnetic rack and discard the solution after it becomes clear.

Note: Magbeads PN should be thoroughly mixed before use. When adding multiple samples to the magnetic beads, it must be shaken several times in between.

2. Remove the centrifuge tube from the magnetic stand and add 500 μL Buffer PGW1 (check whether anhydrous ethanol has been added before use ), vortex mix, and place on a constant temperature mixer at room temperature 1700 rpm shaking mix 2 min. After instant centrifugation, place on a magnetic rack and discard the solution after it becomes clear.

3. Remove the centrifuge tube from the magnetic stand and add 80 μL DNase I mixed solution, vortexed and mixed, and then placed on a thermomixer at room temperature 1700 rpm shake mixing 5 min. After instant centrifugation, place on a magnetic rack and discard the solution after it becomes clear.

4. Remove the centrifuge tube from the magnetic stand and add 700 μL Buffer RW2 (check whether anhydrous ethanol has been added before use ), vortex mix, and place on a constant temperature mixer at room temperature 1700 rpm shaking mix 2 min. After instant centrifugation, place on a magnetic rack and discard the solution after it becomes clear.

5. Repeat step 4 once.

6. Dry at room temperature for 5-10 min to allow the ethanol to fully evaporate. When the surface of the magnetic beads becomes matte and there is no cracking of the beads, add 100 μL RNase - Free Water, use a pipette to blow or shake to mix. After instant centrifugation, place on a constant temperature mixer at 65℃. 1700 rpm Shock 10 min.

Note: After discarding the waste liquid, the centrifuge tube can be centrifuged instantly and placed on a magnetic rack for 10 Use a μL pipette to discard any remaining liquid.

7. Fix the centrifuge tube on the magnetic rack, and after the solution is clarified, transfer the eluted product to a new centrifuge tube and store it at -20℃ for later use. If downstream experiments cannot be performed in time, the eluted product should be stored below -70°C.

## Automated operation (matched with CWE2100)

1. (Continued from Sample Pretreatment) Follow the table below to 96 Add reagents to the DW deep well plate:

| Location | Reagents and dosage |
|---|---|
| 1&7 columns | Supernatant: All<br>Buffer PA: 350 μL<br>Magbeads PN: 20 μL |
| 2&8 columns | Buffer PGW1: 500 μL |
| 3&9 columns | DNase I:80 μL |
| 4&10列 | Buffer RW2:700 μL |
| 5&11列 | Buffer RW2:700 μL |
| 6&12列 | RNase-Free Water:100 μL |

2. Place the 96 DW deep well plate and magnetic rod cover on In the instrument, run the "Plant RNA" program . After the sequence is completed, remove the magnetic sleeve and deep-well plate. Transfer the eluted products in columns 6 and 12 to column 1. .5 mL centrifuge tube (self-prepared) middle, Store at -20℃ for future use. If downstream experiments cannot be performed in time, store the eluted product below -70℃.

## Automated operation

1. (Continued from Sample Pretreatment) Follow the table below to 96 Add reagents to the DW deep well plate:

| Location | Reagents and dosage |
|---|---|
| Plate 1 | Supernatant: All<br>Buffer PA: 350 μL<br>Magbeads PN: 20 μL |
| Plate 2 | Buffer PGW1: 500 μL |
| Plate 3 | DNase I: 80 μL |
| Plate 4 | Buffer RW2: 700 μL |
| Plate 5 | Buffer RW2:700 μL |
| Plate 6 | RNase-Free Water: 100 μL |

2. The 96 DW deep-well plate and magnetic rod set are placed in the CWE960 instrument and the "Plant RNA" procedure. Approximately 35 min post procedure After the run is complete, remove the magnetic sleeve and deep-well plate . The eluted product in 6 was transferred to 1.5 mL centrifuge tube (self-prepared), Store at -20℃ for future use. If downstream experiments cannot be performed in time, store the eluted product below -70℃.