EasyPure® Universal Plant Total RNA Kit

Please read the datasheet carefully prior to use.

Cat. No. ER302

Version No. Version 1.0

Storage: at room temperature (15-25°C) in a dry place for one year; DNase I at -20°C for one year.

Description

The kit is designed for the isolation of RNA from various fresh and dried plant samples, including plant samples rich in polysaccharides and polyphenols. The kit is based on unique extraction system and membrane technology to specifically remove secondary metabolites such as polysaccharides, polyphenols, and lipids in samples, without the need for toxic reagents such as phenol and chloroform. Purified RNA with high quality and good stability can be used for RT-PCR, qRT-PCR, Northern blot, RACE, library construction, etc.

Features

- Wide range of applications: applicable to various plant tissues, especially those rich in polysaccharides, polyphenols or starch
- Fast operation: high-quality genomic RNA can be extracted in less than 30 minutes
- Safe and low toxicity: no toxic organic reagents such as phenol and chloroform
- High purity: the unique technology can efficiently remove impurities such as pigments, polyphenols and polysaccharides in the sample

Kit Contents

Component	ER302-01 (50 rxns)	ER302-02 (200 rxns)
Lysis Buffer 52 (LB52)	40 ml	160 ml
Precipitation Buffer 52 (PB52)	10 ml	40 ml
DNase I (3 units/μl)	1500 U	6000 U
DNase I Reaction Buffer	4×1 ml	15 ml
Clean Buffer 52 (CB52)	20 ml	80 ml
Wash Buffer 52 (WB52)	12 ml	2×22 ml
RNase-free Water	10 ml	20 ml
Filtration Columns with Collection Tubes	50	200
RNA Spin Columns with Collection Tubes II	50	200

Procedures

Add different volumes of absolute ethanol (self-prepared) to CB and WB according to the table below before use.

Component	ER302-01 (50 rxns)	ER302-02 (200 rxns)
Clean Buffer 52 (CB52)	20 ml	80 ml
Wash Buffer 52 (WB52)	48 ml	2×88 ml

1. Weigh about 100 mg of fresh plant tissue or 30 mg of dry weight tissue ground with liquid nitrogen into a 1.5 ml sterile centrifuge tube (self-prepared).

* For samples with high water content, such as apples, tomatoes and other fruits, the initial amount can be increased appropriately.

2. Add 700 μl Lysis Buffer 52 (LB52), mix thoroughly, and incubate at 65°C for 5 minutes.

3. Add 175 µl PB52, mix thoroughly. Centrifuge at 13,500×g for 3 minutes. Transfer all supernatant to Filtration Columns with Collection Tubes (There may be a small amount of precipitation or impurities in the supernatant, which does not affect downstream extraction). Centrifuge at 13,500×g for 2 minutes.

* If the solution is viscous after lysis, add PB52 and place in ice bath for 5 minutes before centrifuging.

4. Add 350 µl of isopropanol (self-prepared) to the above collection tube, mix up and down, and flocculent precipitation may appear at this time.
5. Take all the above mixture and add it to the RNA Spin Columns with Collection Tubes II in two times. Centrifuge at 13,500×g for 30 seconds, and discard the flowthrough.
6. Add 80 µl DNase I working solution to the center of filter membrane in the spin column and keep it still at room temperature for 10 minutes.

* Preparation of DNase I working solution: Add 70 µl DNase I Reaction Buffer into an RNase-free centrifuge tube, then add 10 µl DNase I and mix well.

7. Add 500 µl CB52, centrifuge at 13,500×g for 30 seconds, and discard the flowthrough (check whether absolute ethanol is added to CB before use).
8. Add 500 µl WB52, centrifuge at 13,500×g for 30 seconds, and discard the flowthrough (check whether absolute ethanol is added to WB before use).
9. Repeat step 8 once.
10. Centrifuge at 13,500×g for 2 minutes to completely remove residual WB52. Place the spin column in a new centrifuge tube (self-prepared).
11. Add 50-100 µl RNase-free Water to the center of the spin column. Keep it still at room temperature for 2 minutes. Centrifuge at 13,500×g for 1 minute to elute the RNA.

* To obtain more RNA, repeat step 11 for a second elution. Store RNA solution at -85°C~-65°C.

Notes

- Sample should be fresh or without repeated freezing and thawing.
- The amount of sample should not be too much, otherwise more impurities will be produced, which will block the filter column and spin column and affect the extraction effect.
- Use sterile pipette tips and centrifuge tubes to avoid RNase contamination.
- If Lysis Buffer appears precipitation, dissolve it in a 37°C water bath, and shake well before use.
- Reagents not included in the kit: isopropanol, absolute ethanol and 1.5 ml sterile centrifuge tube.

For research use only, not for clinical diagnosis

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