

## Plant Total RNA Extraction Miniprep System

Isolation of total RNA from 100 mg plant material or  $1 \times 10^7$  cells.

### Kit contents:

RX Buffer (1), PRX Buffer (1), WF Buffer (1), WS Buffer (2), RNase-free ddH<sub>2</sub>O (1), Plant Total RNA Mini Column (250), Shearing Tube (250), Collection Tube (500), 1.5 ml Elution Tube (250) and protocol (1)

### Protocol:

#### <Note>:

1. Add 10  $\mu$ l  $\beta$ -mercaptoethanol ( $\beta$ -ME) per 1 ml RX Buffer or PRX Buffer.
2. Add 180 ml of ethanol (96-100%) to each WS Buffer bottle when first open the bottle.
1. **Grind 100 mg (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.**
2. **Add 450  $\mu$ l of RX Buffer or PRX Buffer ( $\beta$ -ME added) to the tissue powder and vortex vigorously. In most cases RX Buffer is the buffer of choice to lyse plant tissue. However, plant**

**tissues contain sticky secondary metabolites (for example, maize with milky endosperm or mycelia of filamentous fungi), PRX Buffer is used instead.**

3. **Apply lysate to the Shearing Tube sitting in a Collection Tube and centrifuge at full speed (13,000 rpm or 10,000 x g) for 2 minutes. Transfer flow-through sample from the Collection Tube to a new tube.**  
Avoid pipetting any debris and pellet in the collection tube.
4. **Add 230  $\mu$ l (about half of the sample volume) 96-100% ethanol to the clear lysate and mix by pipetting.**  
If sample lysate is lost during the preparation, reduce ethanol volume proportionally.
5. **Apply 680  $\mu$ l of the ethanol added sample (including any precipitate) from step 4 to a Plant Total RNA Mini Column sitting in a Collection Tube, close the cap, centrifuge at 8,000 x g (10,000 rpm) for 1 minute, and discard the filtrate.**  
If the solution remains above the membrane, centrifuge again at 13,000 rpm.
6. **Repeat step 5 for rest of the sample.**
7. **Wash the column once with 0.5 ml of WF Buffer by centrifuging at full speed for 30-60 seconds and discard the filtrate.**
8. **Wash the column twice with 0.7 ml of WS Buffer by centrifuging at full speed for 30-60 seconds and discard the filtrate.**  
Add 180 ml of ethanol (96-100%) to each WS Buffer bottle when first open the bottle.

- 9. Centrifuge at full speed for 3 minutes to remove traces of WS Buffer.**

Residual ethanol may inhibit reverse transcriptase activity.

- 10. Transfer the column to a RNase-free 1.5 ml Elution Tube, add 50  $\mu$ l of RNase-free ddH<sub>2</sub>O, and centrifuge at full speed for 1 - 2 minutes to elute RNA.**

- 11. Store RNA at -70°C.**