User Bulletin

VER.17A

Plant Total RNA Extraction Miniprep System

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

Downstream Application

- Northern blotting
- *In vitro* translation
- cDNA synthesis
- RT-PCR
- NGS
- Gene editing

Product Contents

Cat. No	GPR1001	GPR1002
Preps	50	250
RX Buffer	27ml	135ml
PRX Buffer	27ml	135ml
WF Buffer (RNA)	30ml	150ml
WS Buffer (RNA)	15ml	45ml x2
RNase-free ddH_2O	1.5ml x2	15ml
Plant Total RNA Mini Column	50	250
RNA Collection tube	100	500
RNA Mini Shearing Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Plant Total RNA Extraction Miniprep is shipping and storage at ambient temperature up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37°C to redissolve.

Protocol:

- **Please read the following notes before starting the procedures.**

- **WARNING**, strong acids and oxidants (like for instance bleach) should not be used together with RX buffer (because this kind of reaction would produce cyanide)!

Important Notes

- Add 10 μl β-mercaptoethanol (β-ME) per 1 ml RX Buffer or PRX Buffer.
- For GPR1001 - Add 60 ml of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.
- For GPR1002 - Add 180 ml of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.

Viogene's unique design — EasyLid

The EasyLid is designed to prevent contamination during the procedure.

Twist the arm of the cap and pull the cap to break the EasyLid.

1. Grind 100 mg (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.

2. **Add 450 μl of RX Buffer or PRX Buffer (β-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 2~10 minutes. Transfer flow-through sample from the Collection tube to a new tube.**
   Avoid pipetting any debris and pellet in the collection tube. To centrifuge for 10 minutes will enhance the extracted RNA quality much.

4. **Add 230 μl (about half of the sample volume) 98-100% ethanol to the clear lysate and mix by pipetting.**
   If sample lysate is lost during the preparation, reduce ethanol volume proportionally.

5. **Apply 680 μl 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 60 ml (for GPR1001) or 180 ml (for GPR1002) of ethanol (98-100%) to the 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 tube (not provided), add 50 μl of RNase-free ddH_2O, Stand the column for 5 minutes, and centrifuge for 1-2 minutes to elute DNA. Centrifuge at full speed for 1-2 minutes to elute RNA.**

11. **Store RNA at -70°C.**