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VIOGENE
VER.:12A

User Bulletin

Plant Total RNA Extraction Maxiprep System

Isolation of total RNA from 1 g plant material or 1 x 10^8 cells.

Downstream Application

* Northern blotting
* *In vitro* translation
* cDNA synthesis
* RT-PCR

Product Contents

Cat. No	GPRM1001
Preps	10
RX Buffer	50ml
PRX Buffer	50ml
WF Buffer (RNA)	55ml
WS Buffer (RNA)	25ml
RNase-free ddH_2O	10ml
Plant Total RNA Maxi Column	10
RNA Maxi Shearing Tube	10
Collection Tube	20
Elution Tube	10
Protocol	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Plant Total RNA Extraction Maxiprep is shipped; should be stored at ambient temperature up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37℃ 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

- All centrifugation should be done at room temperature with a swing-bucket centrifuge.
- Add 10 μl β-mercaptoethanol (β-ME) per 1 ml RX Buffer or PRX Buffer.
- Add 100 ml of ethanol (98 ~ 100 %) to the WS Buffer bottle when first open the bottle.

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

2. **Add 4.5 ml 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 (3,000 rpm or 2,500 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 2.3 ml (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 6.8 ml of the ethanol added sample (including any precipitate) from step 4 to a Plant Total RNA Maxi Column sitting in a Collection Tube, close the cap, centrifuge at full speed for 3 minutes, and discard the filtrate.**
   If the solution remains above the membrane, centrifuge again for another 5 minutes.

6. **Repeat step 5 for rest of the sample.**

7. **Wash the column once with 5 ml of WF Buffer by centrifuging at full speed for 3 minutes and discard the filtrate.**

8. **Wash the column twice with 5 ml of WS Buffer by centrifuging at full speed for 3 minutes and discard the filtrate.**
   Add 100 ml 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 15 ml Elution Tube (provided), add 500 μl of RNase-free ddH_2O, Stand the column for 5 minutes. Centrifuge for 1-2 minutes to elute DNA.and centrifuge at full speed for 5 minutes to elute RNA.**

11. **Store RNA at -70°C.**