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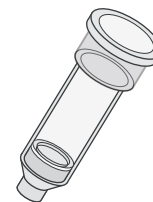
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GenCatch™ Plant Total RNA Extraction Kit



User's Guide for
Total RNA Purification
From Plants

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Plant Total RNA Extraction Miniprep System

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

Kit contents:

RX Buffer (1), PRX Buffer (1), WF Buffer (1), WS Buffer (2), RNase-free ddH₂O (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 μ l β -mercaptoethanol (β -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 μ l 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 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 μ 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 μ 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 μ l of RNase-free ddH₂O, and centrifuge at full speed for 1 - 2 minutes to elute RNA.**
11. **Store RNA at -70°C.**