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Plant/Fungi Total RNA Purification Kit

Supplementary Protocol for Plant Tissue Homogenization Without Liquid Nitrogen Grinding.

Product # 25800, 31350, 25850

1. Lysate preparation

a) Transfer ≤ 50 mg of plant tissue or 5 x 10^6 plant cells into a 2 mL centrifuge tube (not provided). Ensure that plant tissue is about 1 cm x 1 cm in size by either shearing or using a leaf hole punch.
b) Add 600 µL of **Lysis Buffer C**. Vortex vigorously.
c) Incubate sample for 10 minutes at 95°C, inverting 2-3 times throughout to mix.
d) Assemble a **Filter column (clear O-ring)** with one of the provided collection tubes. Pipette the lysate into a Filter Column and spin for 2 minutes at **20,000 x g (~14, 000 RPM)**.
e) Transfer only the clear supernatant from the flowthrough into an RNAase-free microcentrifuge tube (not provided) using a pipette.

Note: Ensure that only the clear supernatant is transferred, avoiding any of the debris at the bottom of the collection tube.

f) Add an equal volume of 96-100% ethanol (provided by the user) to the lysate collected above (100µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

2. Binding to Column

a) Assemble a **Spin Column (grey O-ring)** with one of the provided collection tubes.
b) Apply up to 600 µL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).** Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **20,000 x g (~14,000 RPM)**.

c) Depending on your lysate volume, repeat step **2b** if necessary.

3. DNase Treatment (Optional)

Norgen's Plant/Fungi Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Norgen's RNase-Free DNase I Kit (Product # 25710). This step should be performed at this point in the protocol

4. Column wash

a) Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **20,000 x g (~14,000 RPM)**.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b) Discard the flowthrough and reassemble the spin column with its collection tube.
c) Repeat steps **4a** to **4b** to wash a column a second time.
d) Wash the column for a third time by adding another 400 µL of **Wash Solution A** and centrifuge for 1 minute.
e) Discard the flowthrough and reassemble the spin column with its collection tube.
f) Spin the column for 2 minutes at **20,000 x g (~14,000)** in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a) Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b) Add 50 µL of **Elution Solution A** to the column.
c) Centrifuge for 2 minutes at **200 x g (2,000 RPM)**, followed by a 2 minute spin at **20,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **20,000 x g (14,000 RPM)** for 1 additional minute.
d) **(Optional):** An additional elution may be performed if desired by repeating steps **5b** and **5c**. The total yield can be improved by an additional 20-30% when this second elution is performed.

6. Storage of RNA

The purified RNA sample may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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