

PURIFYING TOTAL RNA FROM PLANT TISSUE OR FILAMENTOUS FUNGI

1 SAMPLE HOMOGENIZATION

See manual for recommended sample amounts and homogenization methods.

2 CELL LYSIS

Add 350 μ L Lysis Buffer RLY and 3.5 μ L β -ME to a maximum of 100 mg ground tissue and vortex vigorously. If lysate solidifies when adding Lysis Buffer RLY, use 350 μ L Lysis Buffer RLS instead.

3 FILTER LYSATE

Place ISOLATE II Filter (violet) in a 2 mL Collection Tube (supplied). Load lysate and centrifuge 1 min at 11,000 x g. Transfer filtrate to a new 1.5 mL microcentrifuge tube (not supplied). If visible pellet forms, transfer supernatant avoiding any pellet to a new 1.5 mL microcentrifuge tube (not supplied).

4 ADJUST RNA BINDING CONDITIONS

Discard ISOLATE II Filter and add 350 μ L ethanol (70%) to homogenized lysate. Mix by pipetting up and down 5 times. Alternatively, transfer flow-through into a new 1.5 mL microcentrifuge tube (not supplied), add 350 μ L ethanol (70%) and mix by vortexing (2 x 5s).

5 BIND RNA

Place ISOLATE II RNA Plant Column (blue) in a 2 mL Collection Tube. Load lysate onto column and centrifuge 30s at 11,000 x g. Place column in a new 2 mL Collection Tube.

6 DESALT SILICA MEMBRANE

Add 350 μ L Membrane Desalting Buffer (MEM). Centrifuge at 11,000 x g for 1 min to dry membrane.

7 DIGEST DNA

Add 10 μ L reconstituted DNase I to 90 μ L Reaction Buffer for DNase I (RDN). Mix by gently flicking tube. Apply 95 μ L DNase I reaction mixture directly onto center of silica membrane. Incubate at room temperature for 15 min.

8 WASH AND DRY SILICA MEMBRANE

1st Wash

- Add 200 μ L Wash Buffer RW1.
Centrifuge 30s at 11,000 x g.
Place column into a new 2 mL Collection Tube.

2nd Wash

- Add 600 μ L Wash Buffer RW2.
Centrifuge 30s at 11,000 x g.
Discard flow-through and place column back into Collection Tube.

3rd Wash

- Add 250 μ L Wash Buffer RW2.
Centrifuge 2 min at 11,000 x g to dry membrane completely.
Place column into a nuclease-free 1.5 mL Collection Tube (supplied).

9 ELUTE RNA

Add 60 μ L RNase-free water (supplied) directly onto center of silica membrane.
Centrifuge at 11,000 x g for 1 min.