PURIFYING TOTAL RNA FROM CULTURED CELLS AND TISSUE

1 **Sample homogenization**
See manual for recommended sample amounts and homogenization methods.

2 **Cell lysis**
Add 350µl Lysis Buffer RLY and 3.5µl ß-ME to cell pellet (up to $5x10^6$ eukaryotic cultured cells) or to ground tissue (up to 30mg tissue) and vortex vigorously.

3 **Filter lysate**
Place ISOLATE II Filter (violet) in a 2ml Collection Tube (supplied).
Load lysate and centrifuge 1 min at 11,000 x g.
If a visible pellet forms, transfer supernatant avoiding any pellet to a new 1.5ml microcentrifuge tube (not supplied).
Alternatively, pass lysate 5-10 times through a nuclease-free 20 gauge (0.9mm) needle and syringe.

4 **Adjust RNA binding conditions**
Discard ISOLATE II Filter and add 350µl ethanol (70%) to homogenized lysate.
Mix by pipetting up and down 5 times.
Alternatively, transfer flow-through into a new 1.5ml microcentrifuge tube (not supplied), add 350µl ethanol (70%) and mix by vortexing (2 x 5s).

5 **Bind RNA**
Place ISOLATE II RNA Mini Column (blue) in a 2ml Collection Tube.
Pipette lysate up and down 2–3 times and load lysate onto column.
Centrifuge 30s at 11,000 x g.
Place column in a new 2ml Collection Tube.

6 **Desalt silica membrane**
Add 350µl Membrane Desalting Buffer (MEM).
Centrifuge at 11,000 x g for 1 min to dry membrane.

7 **Digest DNA**

Add 10µl reconstituted DNase I to 90µl Reaction Buffer for DNase I (RDN).
Mix by gently flicking tube.
Apply 95µl 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 200µl Wash Buffer RW1.
  Centrifuge 30s at 11,000 x g.
  Place column into a new 2ml Collection Tube.

2nd Wash

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

3rd Wash

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

9 **Elute RNA**

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