PURIFYING TOTAL RNA FROM MICRODISSECTED CRYOSECTIONS, LASER CAPTURED CELLS OR CULTURED CELLS

1 **Provide sample**

Transfer sample e.g. microdissected tissue cryosection, pelleted cultured cells (up to 5 x 10^5), or laser captured cells to a sterile 1.5ml microcentrifuge tube (not supplied).

2 **Cell lysis and homogenization**

Add 100µl Lysis Buffer RLY and 2µl TCEP to sample and vortex vigorously (2 x 5s).

3 **Add Carrier RNA**

Add 5µl (20ng) Carrier RNA working solution to lysate.
Mix by vortexing (2 x 5s).
Briefly spin down (1s at 1000 x g).

4 **Filter lysate (optional)**

Place ISOLATE II Filter (violet) in a 2ml Collection Tube (supplied).
Load lysate and centrifuge 30s at 11,000 x g.
Discard ISOLATE II Filter.
Step 4 may be omitted when processing small amounts of sample, e.g. <10^5 cells.

5 **Adjust RNA binding conditions**

Add 100µl ethanol (70%) to homogenized lysate.
Mix by pipetting up and down 5 times.

6 **Bind RNA**

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

7 **Desalt silica membrane**

Add 100µl Membrane Desalting Buffer (MEM).
Centrifuge 30s at 11,000 x g to dry membrane.
Re-use Collection Tube.

8 **Digest DNA**

Add 3µl reconstituted DNase I to 27µl Reaction Buffer for DNase I (RDN).
Mix by gently flicking tube.
Apply 25µl DNase I reaction mixture directly onto center of silica membrane.
Incubate at room temperature for 15 min.

9 **Wash and dry silica membrane**

1st Wash

- Add 100µl Wash Buffer RW1.
  Incubate for 2 min at room temperature.
  Centrifuge 30s at 11,000 x g.
  Place column into a new 2ml Collection Tube.

2nd Wash

- Add 400µl Wash Buffer RW2.
  Centrifuge 30s at 11,000 x g.
  Discard flow-through and place column back into Collection Tube.

3rd Wash

- Add 200µl 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).

10 **Elute RNA**

Add 10µl RNase-free water (supplied) directly onto center of silica membrane.
Centrifuge at 11,000 x g for 30s.