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 1.5 mL microcentrifuge tube (not supplied).

2 CELL LYSIS AND HOMOGENIZATION

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

3 ADD CARRIER RNA

Add 5 µL (20 ng) 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 2 mL 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 100 µL 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 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.

7 DESALT SILICA MEMBRANE

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

8 DIGEST DNA

Add 3 µL reconstituted DNase I to 27 µL Reaction Buffer for DNase I (RDN).
Mix by gently flicking tube.
Apply 25 µL 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 100 µL Wash Buffer RW1.
  Incubate for 2 min at room temperature.
  Centrifuge 30s at 11,000 x g.
  Place column into a new 2 mL Collection Tube.

2nd Wash

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

3rd Wash

- Add 200 µL 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).

10 ELUTE RNA

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