RNA extraction from schistosome intermediate hosts and parasite stages using RNAzol® RT (mRNA isolation)

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Procedure
This protocol yields two RNA fractions; mRNA-containing fraction > 200 bases and microRNA-containing fraction < 200 bases.

1. Homogenization
   • Homogenize the snail, parasite or related tissues using homogenizer with liquid nitrogen
   • Measure the dry weight of homogenized tissues
   • Add RNAzol®RT into dried pack homogenate with ratio1/1 (vol/vol)
   • Mix by vortex

2. DNA/protein precipitation
   • Add 0.4 ml of sterile water into 1 ml homogenate
   • Incubate at room temperature (RT) for 15 min
   • Centrifuge at 12,000 g, RT, 15 min.
   • mRNA precipitation
   • Add 0.4 ml 75% ethanol into 1 ml supernatant
   • Incubate RT for 10 min
   • Spin down at 12,000 g, RT, 8 min

4. mRNA washes
   • Wash pellet with 0.4 ml 75% ethanol with 8,000 g centrifugation for 3 min
   • Repeat washing twice

5. RNA solubilization with water or Formazol®

Total RNA isolation
This protocol yields total RNA comprising all classes of RNA: large nuclear RNA, ribosomal RNA, mRNA, small RNA and microRNA down to 10 bases.

1. Homogenization
   • Homogenize the snail, parasite or related tissues using homogenizer with liquid nitrogen
   • Measure the dry weight of homogenize powder
   • Add RNAzol®RT into dried pack homogenate with ratio1/1 (vol/vol)
   • Mix by vortex

2. DNA/protein precipitation
   • Add 0.4 ml of sterile water into 1 ml homogenate
   • Incubate at room temperature (RT) for 15 min
   • Centrifuge at 12,000 g, RT, 15 min.

3. RNA precipitation
BRI Protocols

RNA extraction protocol

- Add the equal volume of isopropanol
- Incubate RT for 15 min
- Spin down at 12,000 g, RT, 8 min

4. RNA washes
   - Wash pellet with 0.4 ml 75% ethanol with 4,000 g centrifugation for 3 min
   - Repeat washing twice

5. RNA solubilization with nuclease free water or Formazol®

Comments
*The optional purification step using RQI (Promega), 4-bromoanisole (BAN) or other DNase enzyme can be used to further eliminate DNA contamination.
* After solubilization, the procedure for RNA must proceed on ice.
* Keep RNA at minimal temperature at -70 °C

RNAzol product information -

Reference:

NIH NIAID Schistosomiasis Resource Center, 2017