



BIOMEDICAL RESEARCH INSTITUTE

NIH NIAID Schistosomiasis Resource Center

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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 ratio 1/1 (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 ratio 1/1 (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

- 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 RQ1 (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 -

<https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/381/652/r4533bul.pdf>

Reference:

1. Chomczynski P. Reagents and methods for isolation of purified RNA. US Patent 7,794,932 (2010)