RNA Isolation Protocol

James O'Callaghan

Abstract

Citation: James O'Callaghan RNA Isolation Protocol. **protocols.io**
https://www.protocols.io/view/rna-isolation-protocol-htrb6m6
Published: 22 Feb 2018

Protocol

Homogenization

Step 1.

Homogenize up to 100 mg of tissue in 0.7 ml Tri Reagent with zirconia beads using tissue lyser or bead beater.

Phase Separation

Step 2.

1. Pre-spin the 1.5ml Phase lock gel (heavy) tubes at 5000 rpm x 4 min to collect the gel at the bottom of the tube.

2. Add the cell homogenate to the phase lock gel for 10 min at room temp.

3. Add 0.27 ml of chloroform per tube. **Cap and shake vigorously for 15 seconds**. DO NOT VORTEX.

4. Centrifuge at 12,000 RPM for 10 minutes at Room Temperature.

RNA Precipitation

Step 3.

1. Clear, aqueous phase should be on top of the phase lock gel. Phenol Chloroform phase should be below the gel. If top layer is gel, break with pipet tip and then remove clear phase without breaking bottom gel layer. Pink layer should remain on bottom.

2. Transfer the aqueous phase to a fresh microfuge tube.

RNA Wash

Step 4.

1. Add 175 ul of 100% ethanol to the supernatant. – DROP WISE WHILE SLOW VORTEXING.

2. Vortex and then add this mixture to an RNeasy Column.

Rneasy Column Purification

Step 5.

1. Centrifuge at 12,000rpm for 15 seconds at room temperature. Pour the flow through back into the column and centrifuge again. Discard final flow through after 2 passes.

2. Add 700 ul of buffer RW1 from the Qiagen Kit. Spin at 12000 rpm for 15 seconds at Room temperature. Discard flow through and collection tube.

3. Prep RPE buffer from the Qiagen Kit.. 4 Parts Ethanol to 1 part RPE buffer…i.e (20 mls Ethanol to 5 mls RPE).

4. Transfer the RNeasy Column into a new 2ml collection tube. Prior to this step, add ethanol to RPE buffer (amount needed daily) if not already done. Add 500 ul of RPE buffer. Centrifuge at 12,000 RPM for 15 seconds. Discard flow through.

5. Add another 500 ul of RPE buffer onto the column. Spin at 12,000 RPM for 2 minutes at Room Temperature. Discard flow through, pop caps, and do quick spin to remove all ethanol.

6. Transfer column to a 1.5 ml tube from the Qiagen kit. Pipet 15 – 25 ul (based upon starting tissue) of nuclease free water directly onto the column membrane. Cap the tube and allow to stand at RT for 10 minutes. Centrifuge at 14,000 rpm for 2 min.

7. Add another 10 – 25 ul water onto the column and repeat centrifugation. Store at -75 degrees C.