RNA Isolation from Tissue Culture Cells

Use Qiagen Rneasy Midi/Maxi Kit

Before using kit:
Add BME to buffer RLT, 10 ul BME per 1 ml buffer RLT (stable for 1 month after addition)

Add 4 volumes of ethanol to buffer RPE, as indicated on bottle.

Do not use refrigerated centrifuge – don't allow to cool below 20C during spins.

1. **Harvest cells**:

   If growing in a monolayer, cells can be lysed directly in the plate or trypsinized and collected as a cell pellet prior to lysis.

   To lyse directly in cell culture plate: Determine approximate number of cells (75mm = 1×10^7). Completely aspirate cell culture media and continue with step 2 of protocol.

   If growing in a flask, trypsinize cells. Determine number of cells (T75 is approx. 1×10^7). Aspirate media and wash cells in PBS. Aspirate PBS and add trypsin. When cells detach, add media to inactivate trypsin and transfer cells to RNase free tube and pellet at 300 g for 5 minutes. Completely aspirate supernatant and proceed with step 2.

2. Disrupt cells in Buffer RLT. For 5×10^6 – 3×10^7 cells use 2.0 ml RLT, up to 1×10^8 cells, use 4 ml RLT, if more cells use Maxi column.
3. Homogenize cells using tissuemizer at least 45 seconds(or vortex then pass lysate 5-10 times through 18-20 gauge needle).
4. Add 1 volume (2.0 or 4.0 m l) 70% ethanol and mix thoroughly by shaking vigorously.
5. Apply sample to RNeasy column. Centrifuge 3000-5000g for 5 min. Discard flow through, reuse centrifuge tube.
6. Add 4.0 ml of Buffer RW1 to column. Centrifuge 3000-5000g for 5 min. Discard flow through.
7. Add 2.5 ml Buffer RPE to column and centrifuge 3000-5000g for 2 min. Discard flow through. Reuse centrifuge tube.
8. Add another 2.5 ml Buffer RPE to column. Centrifuge 3000-5000g for 5 min.
9. Transfer column to new 15 ml collection tube. Pipet 150-250 ul RNase-free water directly onto membrane. Let stand 1 minute, centrifuge 3000-5000g for 3 min.
10. Repeat elution step with a second volume of RNase free water.