Protocol: RNA Extraction from Tissue Culture Cells

We use the Qiagen RNeasy kit for RNA extraction and purification from tissue culture cells. This protocol is adapted from the Qiagen RNeasy Quick Start Protocol.

The volume of Buffer RLT (lysis buffer) depends upon the size of the dish. Assuming that RNA extraction will be performed on nearly confluent tissue culture dishes, use the following table to determine the amount of Buffer RLT needed for lysis.

NOTE: **Perform all RNA work under RNA-clean working conditions and use designated RNA Only filter tips, plastics, and solutions! Clean pipettes, tube racks, tip boxes, and pens thoroughly with RNase Zap before commencing work.**

Sample	Number of Cells	Volume of Buffer RLT
6-well plate or 60mm dish	$<5 \times 10^6$ cells	350μl RLT
100mm dish	$<1 \times 10^7$ cells	600μl RLT

1. Determine the volume of Buffer RLT you will need, based on the number and size of dishes to be extracted, round up to the nearest ml, and transfer to a conical tube. Add 10μl β-mercaptoethanol (BME) per ml to Buffer RLT and vortex.
2. Bring Buffer RLT (+BME) and RNA-clean microcentrifuge tubes to the TC hood.
3. Remove old media and wash in PBS. Aspirate the PBS and add the appropriate volume of Buffer RLT (+BME) to each well or dish. Using a cell scraper, thoroughly collect the lysate and transfer to a labeled microcentrifuge tube.
4. Back at the bench, add one volume of RNA-clean 70% ethanol to each sample. Mix by pipetting up and down several times.
5. Transfer 700μl at a time to the RNeasy spin column. Centrifuge at 8,000 x g for 20 seconds. Discard the flow-through. Repeat if necessary (if additional volume remains).
6. Add 350μl RW1 Buffer to the column. Centrifuge at 8,000 x g for 20 seconds. Discard the flow-through.
7. Create a DNase working solution by adding 10μl DNase (Qiagen RNase-free DNase Set) to 70μl Buffer RDD for each RNA purification column (plus one extra reaction so that we have extra volume to work with). Note: do not re-freeze DNase. Discard unused enzyme.
8. Pipette 80μl of the DNase working solution directly onto the white membrane inside the spin column. Incubate on the bench top for 15 minutes.
9. Add 350μl Buffer RW1 to the spin column. Centrifuge 8,000 x g for 20 seconds. Discard the flow-through.
10. Add 500μl Buffer RPE to the spin column. Centrifuge 8,000 x g for 20 seconds. Discard the flow-through.
11. Add 500μl Buffer RPE to the spin column. Centrifuge 8,000 x g for 2 minutes. Discard the flow-through.

12. Transfer the spin column to a new collection tube (provided in kit). Centrifuge at full speed for 1 minute.

13. Transfer the spin column to a 1.5ml microcentrifuge tube (provided in kit). Add 50µl RNase-free Water (provided in kit) directly to the white membrane in the spin column. Incubate on the bench top for 3-5 minutes. Centrifuge at 8,000 x g for 1 minute.

14. Analyze concentration of each RNA extraction using the Nanodrop One^{C}. RNA is stored at -80°C.