RNA Isolation and Purification from Cell Lines Using Qiagen's RNA Mini Kit

Materials:

1.) RNeasy Mini Kit (Qiagen #74106)

2.) QIAshredder Columns (Qiagen #79656)

3.) B-Mercaptoethanol (Sigma #M6250-100ML)

4.) Nanodrop or Spectrophotometer

5.) 1.5mL RNase Free Microfuge Tubes (Fisher # NC9445663)

6.) 70% Ethanol

Before Starting:

1.) Add B-Mercaptoethanol to Buffer RLT before use. Aliquot the amount of Buffer RLT needed into a 15mL centrifuge tube. For every 1mL of Buffer, add 10ul of B-Me.

2.) Add 44mL of 100% Ethanol to Buffer RPE (if this has not already been done).

Notes:

1.) B-Mercaptoethanol, Buffer RLT, Buffer RW1 and Buffer RPE need to be collected as hazardous waste. They can not go down the drain!

2.) RNA is easily degraded. Be sure the work space is clean and has been wiped with RNase Zap or a similar product. Work quickly and place the RNA in the -80C as quickly as possible after isolation and purification.

Procedure:

Note: The cell pellet from a confluent 10cm plate is too large for one column and will result in significant RNA loss. Add 1200ul of Buffer RLT to the pellet and then split this volume between two QiaShredder columns and two RNeasy columns. Each column will therefore get 600ul of lysate. After elution, combine the two tubes into one before taking an OD.

1.) Harvest cells according to standard tissue culture procedures to obtain a cell pellet

2.) To the cell pellet, add 1200ul of Buffer RLT (containing B-Me)

3.) Vortex the pellet to disrupt it as much as possible

4.) Divide the 1200ul of lysate between two QiaShredder columns, so that each column has 600ul of lysate.

5.) Centrifuge at maximum speed for 2 minutes to fully homogenize the cells

6.) Add 600ul of 70% Ethanol to each tube of the homogenized cells and mix well by pipet. The total volume in each tube will now be 1200ul.

Note: Pipet well to mix – a precipitate will most likely form

7.) Pipet 600ul of the sample in each tube onto RNeasy mini columns (including the precipitate)

8.) Centrifuge at 11,000rpm for 30 seconds. Discard the flow through (collect as hazardous waste)

9.) Add the remaining 600ul to the columns (including the precipitate)

10.) Centrifuge at 11,000rpm for 30 seconds. Discard the flow through (collect as hazardous waste)

11.) Add 700ul of Buffer RW1 to the columns.

12.) Centrifuge at 11,000rpm for 30 seconds. Discard the flow through (collect as hazardous waste).

13.) Transfer the RNeasy filters into fresh 2mL collection tubes

14.) Add 500ul of buffer RPE to the columns.

15.) Centrifuge at 11,000rpm for 30 seconds. Discard the flow through (collect as hazardous waste).

16.) Add another 500ul of Buffer RPE to the columns.

17.) Centrifuge at 11,000rpm for 2 minutes. Discard the flow through (collect as hazardous waste).

18.) Transfer the RNeasy columns to fresh 2mL collection tubes

19.) Centrifuge at 11,000rpm for 1 minute. Discard the flow through (collect as hazardous waste)

20.) Transfer the RNeasy columns into fresh 1.5mL nucleic acid free microfuge tubes.

21.) Add 30ul of RNase free water directly onto the columns

22.) Centrifuge at 11,000rpm for 1 minute

23.) Add another 30ul of RNase free water directly onto the columns

24.) Centrifuge at 11,000rpm for 1 minute

25.) Combine the two tubes into one tube for a total of 120ul.

26.) Quantitate the RNA on the Nanodrop.

Note: A260/280 ratios should be between 1.9 and 2.1. If they are not, additional cleanup using the RNeasy mini columns is necessary