

## RNA PREPARATION FOR MICROARRAY

### HARVEST CELLS

Cells grown in suspension

Determine number of cells.

Pellet cells for 5 min., 300 x g, 4°C.

Carefully remove supernatant by aspiration.

Resuspend cell pellet in ice cold 1X PBS.

Pellet cells for 5 min., 300 x g, 4°C.

Carefully remove supernatant by aspiration.

Continue with RNA preparation or snap freeze the cell pellet in liquid nitrogen and store, -70°C.

Cells grown in monolayer

Remove growth media. Add 5ml/dish ice cold 1X PBS. Scrape cells.

Pool scraped cells in cold PBS into polypropylene tube(s), sitting on ice.

Determine number of cells.

Pellet cells for 5 min., 300 x g, 4°C.

Resuspend cell pellet in ice cold 1X PBS.

Pellet cells for 5 min., 300 x g, 4°C.

Carefully remove supernatant by aspiration.

Continue with RNA preparation or snap freeze the cell pellet in liquid nitrogen and store, -70°C.

### RNA PREPARATION

Notes: Refer to Qiagen handbook for cell types and overloading columns.

Freshly prepare Buffer RLT by adding 10 ul

-ME/ml buffer (4 ml of Buffer RLT will be used per column)

All steps for the extraction of RNA are performed at room temperature. All centrifugations are carried out using a swinging bucket rotor at 4000 x g (the maximum speed of 3500-5000 rpm corresponds to 3000-5000 x g for most rotors.)

Hold pellets on ice. Frozen pellets will need to be thawed a little. To lyse the cells, loosen cell pellet thoroughly by flicking the tube. Add 4 ml of Buffer RLT plus □-ME per column to be run.

Homogenize cells.

Repeat until the solution is completely clear. Qiagen states that samples can be stored in this state for several months at -70°C.

Add an equal volume of 70% room temperature ethanol to the homogenized lysate and mix by shaking or vortexing. Do NOT centrifuge.

Apply 4 ml of the sample to an RNeasy midi-spin column sitting in a 15 ml (supplied) centrifuge tube. Close the tube lightly. Spin for 3 minutes. Discard the flow-through.

Repeat with the remaining lysate, if there was more than 4 ml. Re-use the tube in step 5.

Add 4 ml of Buffer RWI to each column, close the centrifuge tube lightly and spin 3 minutes to wash the column. Discard the flow-through. Re-use tube in step 6.

Add 2.5 ml of 1X Buffer RPE to the column, close lightly and spin 2 min. Discard flow-

through.

Add another 2.5 ml of 1X Buffer RPE to the column, close tube lightly and spin 10 min. to dry the column membrane.

To elute, transfer RNeasy midi-spin column to a new 15 ml collection tube (supplied). Pipet 200µl of RNase-free water directly onto the column membrane. Close the tube lightly, let it stand for 1 minute and spin for 3 min.

Add another 150µl of RNase-free water and respin 5 min. Mix and transfer the eluted RNA to a sterile 1.5 ml microfuge tube.

Quantitate, concentrate and store RNA in a microcentrifuge tube at -70°C.

### QUANTITATE the RNA

Use a 100µl glass cuvette. Dilute 1µl of RNA with 249µl of RNase-free water (1:250). Blank with the same water. Read the OD at 260 and 280. A 260/280 ratio of 1.6 to 1.7 is good for RNA in water (it should be higher if in buffer). Abs at 260 x 10 = µg/µl.

#### BEFORE CONCENTRATION

~Final  
RNA vol.(µl) P.C. (µg/µl)

#### AFTER CONCENTRATION

estimated  
µg RNA P.C. (µg/µl) Final  
vol.(µl) Total (µg)

### CHECK QUALITY OF RNA on a gel

Set up a 1.2% Formaldehyde Gel. Run 3 □g of RNA

per lane. For a mini-gel with 8 wells:

0.3 g agarose

18 ml s d water

Microwave to dissolve the agarose.

Add 2.5 ml 10X FA Buffer

4.45 ml formaldehyde

0.075 µg EtBr/ml of gel Allow to cool to about 60°C. Pour into the gel box with a comb.

Add 1X FA Buffer to the gel box once the gel has set. Allow gel to equilibrate with buffer for about 30 min.

Samples:

3  $\mu\text{g}$  RNA \_\_\_\_\_  $\mu\text{l}$   
water to 8  $\mu\text{l}$  \_\_\_\_\_  $\mu\text{l}$   
5X FA RNA Loading Buffer 2  $\mu\text{l}$  to each

Denature samples at 60°C for 10 minutes; sit on ice 2 - 5 minutes; briefly spin down; load. Run at 100 V for 0.8 - 0.9 hr.

Photograph the gel on settings 8 and 2, orange filter, with Type 57 Polaroid film. 2 bands should be seen, 18 and 28 kD.

### CONCENTRATION AND STORAGE OF RNA FOR MICROARRAY

Concentrate with a pre-wetted Microcon-30 filter unit (Amicon #42410) to a final concentration of approximately 1.1 - 5.0  $\mu\text{g}/\mu\text{l}$ . Spin at 11,000 rpm, room temp. Total spin time will vary according to how much starting sample you have and % reduction desired. For example, if the starting volume is  $\geq 500\mu\text{l}$  and you want about an 85% reduction in volume, start with a 12 minute spin. Add time as needed. The waste volume can be measured to estimate how much your final RNA volume is in the column unit. When at a desired volume, invert the column unit over a clean tube and spin 2 minutes at 11,000 rpm. Rotate and spin again.

Recheck the ODs using 0.5 $\mu\text{l}$  of RNA in 249.5 $\mu\text{l}$  of sterile water.

For NIEHS In-House Produced Arrays:

Aliquots of 100 $\mu\text{g}$  should be stored at 2 $\mu\text{g}/\mu\text{l}$  in RNase-free water.

For Agilent Commercial Arrays:

Aliquots of 60  $\mu\text{g}$  should be stored between 1.1 - 5.0  $\mu\text{g}/\mu\text{l}$  in RNase-free water.

RNA should be snap frozen and stored at -80°C or over liquid nitrogen in a LN<sub>2</sub> freezer. Keep on ice when pulled out to use.