RNAeasy Mini protocol for RNA extraction from cells

1. Just before using RLT buffer add 10 µl of B-ME per ml of RLT buffer.
2. Pellet desired cells by centrifugation at 1400 rpm for 6 mins at 4°C. Loose cells by flicking gently. Add RLT buffer containing BME to the cell pellet.
   For cells upto 5x 10^6 cells add 400 µl of RLT buffer, for upto $1x10^7$ cells add 600 µl of RLT buffer. Vortex gently or pipette up and down to mix well. At this point the lysate can be stored in –70°C and processed later
3. Thaw the RNA lysate on ice to proceed with RNA extraction.

**** Label microcentrifuge tubes while RNA lysate is thawing. ****

a. For cells up to $<1x10^5$ cells homogenization can be carried out by vortexing for 1 min

b. For higher cell concentration pipette lysate directly into a 1.5ml tube and use pestle to homogenize.

or

c. Pass lysate 5 times through a 20G needle fitted to a syringe.

4. Add 1 volume of 70% ethanol (usually 400 µl or 600 µl) to the homogenized lysate (flow through from the QIAShredder step 3b). Mix well by pipetting.
5. Apply 700 µl of the sample including any precipitate to an RNEasy column sitting in a 2 ml collection tube. Spin for 30 sec at 10,000rpm (*14k per AKS). If volume exceeds 700 µl, successively load aliquots onto the RNEasy colum and centrifuge again. Reuse the collection tube but discard flow-through after each step. Reuse collection tube in the next step.
6. Pipette 700 µl of Buffer RW1 onto the RNEasy column and centrifuge for 30 sec at *14,000rpm (once it has reached proper speed) to wash. Discard flow through and collection tube.
7. Transfer RNEasy column into a 2ml collection tube (supplied). (Make sure that ethanol has been added to Buffer RPE). Pipette 500 µl Buffer RPE onto the RNEasy column, and centrifuge for 30 sec at *14,000rpm to wash. Discard flow through and reuse the collection tube in step 8.

8. Pipette 500 µl Buffer RPE onto the RNEasy column. Centrifuge for 2 min at maximum speed (14k) to dry the RNEasy membrane.
9. Place the RNEasy spin column in a new 2ml or 1.5 ml collection tube (not supplied) and discard the collection tube with the filtrate. Spin at 14,000rpm for 1 min.
10. Transfer the RNEasy column into a new 1.5 ml collection tube (supplied) and pipette 30 µl of RNAse free water directly on the RNEasy membrane. Centrifuge for 1 min at 14,000 rpm to elute. Repeat this step with the same eluted RNA to increase yields.

<u>For each sample</u> to be processed you will need the following:

1 QIAshredder column labeled on top and side with appropriate sample number
1 RNeasy column labeled on top with sample number
1 2mL round bottom collection tube unlabeled (provided)
1 1.5mL microcentrifuge tube with top removed unlabeled (not provided)
1 1.5mL final collection tube (provided) labeled with sample number and RNA on top; experiment number, sample number and RNA on side.

Different tubes should be housed in separate racks to avoid confusion when processing. Make your own working aliquots of the following reagents for each isolation. Be sure to prepare for 1-2 additional reactions to allow for loss due to pipetting.

600uL 70% ethanol made with DEPC water
700uL RW1 buffer (provided with kit)
1mL RPE buffer (provided with kit)
30uL RNase free water (provided with kit)