RNA Isolation Protocol using the Qiagen RNeasy Mini Kit

Materials and preparation of solutions:

- RNeasy spin columns
- Collection tubes: 1.5 ml; 2ml
- Pipettes with filter
  20-gauge needle (0.9 mm diameter)
- Buffer RLT (lysis buffer) (contains guanidine salt)
- Buffer RW1
- Buffer RPE concentrate (before using for the first time, add 4 volumes of ethanol (96-100%) to obtain a work solution)
- RNase-free water
- beta mercaptoethanol (βME)
- DEPC (Diethylpyrocarbonate) (from Sigma, cat. nr. D-5758)
- 0.1% DEPC (Diethylpyrocarbonate) H_2O: mix 1ml DEPC in 1000 ml H_2O and autoclave.
- 70% ethanol: 7ml abs. ethanol + 3ml aut. DEPC H_2O

RNA isolation:

- RNases are everywhere. First we have to be sure we have eliminated them. Important is to perform RNA isolation under a sterile hood or in a lab where only RNA isolation is performed (if possible.) If this is not possible, we have to try to get rid of RNase as much as possible. Important is to use pipettes, tips and tubes only for RNA isolation and manipulation.
- RNase elimination: chloroform and aut. DEPC H_2O (cleaning the table and tools). Skin is a very rich source of RNase, be sure to wear gloves during the procedure.
- Cells (samples) are harvested and frozen in aliquots

1. Bring the sample aliquots to room temperature by adding 350 µl of RLT (lysis) buffer + 3,5 µl of βME
2. Mix by pipetting
3. Homogenize the sample: pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter)
4. Add 350 µl 70% ethanol to the sample and mix well by pipetting
5. Transfer the 700 µl of sample, including any precipitate that may have formed, to RNeasy spin column
6. Centrifuge 30 sec. at 13000 rpm
7. Discard the flow-through liquid. Your RNA is on the column now.

8. Add 700 µl Buffer RW1 to RNeasy spin column
9. Centrifuge 30 sec. at 13000 rpm
10. Discard the flow-through liquid.
11. Add 500 µl RPE buffer to RNeasy spin column
12. Centrifuge 30 sec. at 13000 rpm
13. Discard the flow-through liquid.
14. Add 500 µl RPE buffer to RNeasy spin column
15. Centrifuge 2 min. at 13000 rpm
16. Discard the flow-through liquid and place the column in a new clean 2 ml tube.
17. Centrifuge 1 min at 13000 rpm
18. Place the column in a new clean 1.5 ml tube.
19. Add 30-50 µl of RNase free water onto the membrane of the column
20. Centrifuge 1 min. at 13000 rpm. Now, your RNA is at the bottom of the tube.
21. In order to recover as much RNA as possible, collect the water with RNA from the tube and re-add it to the membrane
22. Centrifuge 1 min. at 13000 rpm

<u>Quality and quantity control:</u> using a Multi-Mode Microplate Reader

- Drop 2 x 2 µl RNase-free water (as Blank) on the first row, and 2 x 2 µl RNA on the second row of microplate insert (Take3).

- Measure the sample on 260/280 nm using the Gene5. program /Nucleic Acid Quantification./

- Measure A_{260} and A_{280} of the RNA solution. Calculate the RNA content assuming that one A_{260} unit equals 50 µg of double-stranded RNA per ml.

  The A_{260}/A_{280} ratio of the isolated RNA should be within the 2-2.2 range.

Quality control: run the RNA on an agarose gel.

Store the RNA at -20^{o}C several weeks or at -80 ^{o}C for long time.