RNeasy Powersoil Total RNA Kit

- **Add 2.5 ml bead solution** (also in the cold room) work @ RT
- **0.25 ml Solution SR1**
- **0.8 ml of Solution IRS**
  Note: volume now is ca. 4 mL
- **Add 2g samples** to the 15 ml bead tube (provided)
  Note: work on metal plate pre-cooled at -80 C (at least for 3-4 hours), and open tube in a petri dish; add sediments to extraction tube to reach ca. 6 mL.
- **Add 3.5 ml Phenol/chloroform/isoamyl** alcohol.
- Cap and vortex the bead tube to mix until the biphasic layer disappears.
- Place the bead tube on a vortex adapter and **vortex at maximum speed for 20 min**.
- Remove the bead tube and **centrifuge ad 3000x g for 10 min.**
- **Transfer the upper phase** to a clean 15 ml collection tube (provided). Discard the phenol/chloroform/isoamyl alcohol.
- **Add 1.5 ml of solution SR3** to the aqueous phase and vortex to mix.
- **Incubate at 2-8°C for 10 min**
- **centrifuge at 2500x g for 10 min at RT**
- **Transfer the supernatant**, without disturbing the pellet, to a new 15 ml collection tube (provided)
- **Add 1:1 vol of Solution SR4 (ca. 5-6 ml)** to the supernatant in the collection tube and invert or vortex to mix.
- **Incubate at -20°C for 1 h.**
- **Centrifuge at 2500x g for 30 min**.
- **Decant the supernatant** and invert the 15 ml collection tube on a paper towel for 5 min.
- Shake solution **SR5 to mix and add 1 m**l to the 15 ml collection tube. Resuspend the pellet completely by repeatedly pipetting or vortexing
- Prepare one **mini column** for each RNA isolation sample:
  - Remove the cap of a 15 ml collection tube and place the RNA capture column inside it. The column will hang in the collection tube
  - **Add 2 ml of Solution SR5** to the mini column. Allow it to completely gravity flow through the column and collect in the 15 ml tube.
- **Add RNA isolation sample** from step 12 onto the mini column and allow it to gravity flow through the column into the 15 ml collection tube.
- **Add 1 ml of solution SR5** to the mini column and allow it to completely gravity flow into the 15 ml collection tube
- **Transfer the mini column** to a new 15 ml collection tube.
- Shake solution **SR6 to mix and then add 1 ml** to the mini column to elute the bound RNA. Allow solution SR6 to gravity flow into the 15 ml collection tube
- **Transfer the eluted RNA to a 2.2 ml collection tube**.
- **Add 1 ml of solution SR4**. Invert at least once to mix and
- **Incubate overnight at -80°C and then 30 min at -20°C – or 1 hours at -20°C**
- Centrifuge the 2.2 ml collection tube at **13000x g for 15 min** to pellet the RNA.
- **Decant the supernatant** and invert the 2.2 ml collection tube onto a paper towel for **10 min to air dry pellet**
- **Resuspend the RNA pellet in 50 µl of solution SR7 and transfer in a PCR tube (0.5 mL)**
  Note: This is a potential stopping point. Store the sample at −80 °C.

Procedure for DNA removing with TURBO DNA-*free* Kit (ambion)

Note: pre-heat the waterbath at 37°C

- add 5 µl (0.1 volume) of **10X TURBO DNase Buffer** and 1.5 µl of **TURBO DNase** to the RNA, and mix gently (flicking the bottom of the tube)
- incubate at 37°C for 20 min in pre-heated waterbath
- Remove the mixture from the waterbath, and add an additional 1.5 µL of TurboDNase.
- Return to the waterbath for another 20 min.
- add 10 µl (0.2 volume) of resuspended **DNase Inactivation Reagent** and mix well, incubate 2-5 min at room temperature (22-26°C) and mix (2-3 times by flicking), vortexing every 20 or 30 s

Note: if DNase Inactivation Reagent is difficult to pipette see Kit instruction!

- centrifuge at 10,000 rpm for 1.5 min (set 2 min)
- Being careful not to disturb the inactivation reagent at the bottom of the tube, transfer the supernatant (~50-60 µL) to a new V-collection tube and place on ice.
- adjust volume to 100 µL with 40-50 µL of RNase free water (e.g. use that provided in Clean up kit)

Note: This is a potential stopping point. Store the sample at −80 °C.

Procedure for RNA purification and concentration with RNeasy MinElute Clean up kit (Qiagen)

Note: as final eluent solution use 30 µl of **RNA Secure 1x solution** (ambion), pre-heat the solution at 60°C, and let cool down at room temperature right before use (see kit instruction).

- to 100 µl of RNA add 350 µl **Buffer RTL**, and mix well
- add 250 µl of **96-100% ethanol** and mix well by pipetting
- transfer the sample (700 µl) to a RNeasy MinElute spin column placed in 2 ml collection tube (supplied), close the lit and centrifuge for 15 sec (set 20 sec) at 10,000 rpm
- discard the flow-through
- place the RNeasy MiniElute spin column in a new 2 ml collection tube (supplied), and add 500 µl **Buffer RPE**, centrifuge for 15 sec (set 20 sec) at 10,000 rpm
- discard the flow-through

-add 500 µl **80% ethanol** (prefiltered) to a RNeasy MinElute spin column, close the lit and centrifuge for 2 min (set 2:30 min) at 10,000 rpm

- discard the flow-through and collection tube
- place the RNeasy MiniElute spin column in a new 2 ml collection tube (supplied), open the lit of the spin column and centrifuge for 5 min at 10,000 rpm
- discard the flow-through and collection tube
- place the RNeasy MiniElute spin column in a new 1.5 ml collection tube (supplied), add 30 µl of **RNA Secure 1x solution**, centrifuge for 1 min (set 1:30 min) at 10,000 rpm, repeat
- pass the flow-through (eluent+RNA) a second time on the same spin column, make sure the liquid is over the filter before centrifugation
- store RNA at -80°C.