Post-BioAnalyzer DNase Treatment and Cleanup of RNA

Note: RNeasy Mini Protocol for RNA Cleanup should be used on samples that the NMG requests **DNase treatment of samples after QC of RNA

Qiagen RNeasy Mini Kit – Cat # 74903 or 74904
RNase-Free DNase Set – Qiagen Cat# 79254 (50 digestions)

- a maximum of 100µg RNA can be used in the RNA cleanup protocol
- add ß-ME to Buffer RLT before use (10µl/ml RLT)
- prepare DNase I stock solution before using for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl RNase-free water provided. Mix gently by inverting the tube. **Do not vortex**.
- for long-term storage of DNase I, divide it into single-use aliquots, and store at –20°C for up to 9 months. Do not refreeze the aliquots after thawing.
- perform all steps of the protocol at RT. During the procedure, work quickly.
- all centrifugation steps are performed at 20-25°C, ≥10,000 rpm.

1. Adjust sample to a volume of 100µl with **RNase-free water**.
2. Add **350µl Buffer RLT** and mix thoroughly.
3. Add **250µl 96-100% EtOH** to the diluted RNA, and mix thoroughly by pipetting.
4. Apply the **sample (700µl) to an RNeasy mini column** placed in a 2ml collection tube (supplied). Centrifuge **15 s**, ≥10,000 rpm. Discard the flow-through and collection tube.
5. Transfer column to a new 2 ml collection tube. Pipet **350 µl Buffer RW1** (Buffer **BR3**, if blood RNA) into column, and centrifuge for **15 s** at ≥10,000 rpm. Discard the flow-through.
6. Add 10 µl DNase I stock solution to 70 µl Buffer RDD for each digestion (example: for 10 samples, add 100 µl DNase I stock solution to 700 µl Buffer RDD). Mix by **gently flicking** the tube, and centrifuge briefly to collect residual liquid from the sides of the tube. **Do not vortex.**
7. Pipet **80µl DNase I** incubation mix directly onto the spin-column membrane, and place on the benchtop (20-30°C) for **15 min**.
8. Pipet **350µl Buffer RW1** (or **BR3**, if blood RNA) into column. Centrifuge for **15 s**, ≥10,000 rpm. Discard the flow-through.
9. Pipet **500µl Buffer RPE** into column. Centrifuge **15 s**, ≥10,000 rpm. Discard the flow-through.
10. Add another **500µl Buffer RPE** onto the column. Centrifuge **2 min**, ≥10,000 rpm.
11. Transfer the RNeasy column to a new 2 ml collection tube. Centrifuge **1 min**, full speed.
12. Transfer the RNeasy column to a new 1.5 ml collection tube (supplied). To **elute**, pipet **30 - 50µl RNase-free water** directly onto the RNeasy silica-gel membrane. Centrifuge **1 min**, ≥10,000 rpm.
13. If the expected RNA yield is >30µg, repeat the elution step (step 8) with a 2nd volume of RNase-free water. Elute into the same collection tube.
    - To obtain a higher total RNA concentration, use the 1st eluate in this 2nd elution step (pipet the 1st eluate from the bottom of the collection tube and pipet it directly onto the RNeasy silica-gel membrane). Centrifuge 1 min, ≥10,000 rpm.
14. Measure the concentration and 260/280 on the NanoDrop and record.