

## Post-BioAnalyzer RNA Clean-up

\*\*Note: RNeasy Mini Protocol for RNA Cleanup should be used on samples that the NMG requests **cleanup** of samples after QC of RNA, if on-column DNase treatment was performed initially

### Qiagen RNeasy Mini Kit

\*a maximum of 100µg RNA can be used in the RNA cleanup protocol.

\*add β-ME to Buffer RLT before use (10µl/ml RLT)

\*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). Close the tube gently, and centrifuge **15 s**, ≥10,000 rpm. Discard the flow-through and collection tube.
5. Transfer the RNeasy column to a new 2 ml collection tube (supplied). Pipet **500µl Buffer RPE** onto the column. Close the tube gently, and centrifuge **15 s**, ≥10,000 rpm. Discard the flow-through.
6. Add another **500µl Buffer RPE** onto the column. Close the tube gently, and centrifuge **2 min**, ≥10,000 rpm to dry the membrane.
7. Transfer the RNeasy column to a new 2 ml collection tube. Centrifuge 1 min, ≥13,000 rpm.
8. 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. Close the tube gently, and centrifuge 1 min, ≥10,000 rpm.
9. If the expected RNA yield is >30µg, repeat the elution step (step 8) with a 2<sup>nd</sup> volume of RNase-free water. Elute into the same collection tube.
  - To obtain a higher total RNA concentration, use the 1<sup>st</sup> eluate in this 2<sup>nd</sup> elution step (pipet the 1<sup>st</sup> eluate from the bottom of the collection tube and pipet it directly onto the RNeasy silica-gel membrane). Centrifuge 1 min, ≥10,000 rpm.
10. Measure the concentration and 260/280 on the NanoDrop and record.