Clean Up of RNA samples Using The Total RNA Kit I (R6834)

1. Transfer 100 µl of the RNA Sample to a 1.5 mL Centrifuge Tube. If the sample is less than 100 µl bring up the sample volume to 100 µl using Nuclease Free Water.

2. Add 100 µl of TRK Lysis buffer to the sample and mix by pipetting or inverting.

3. **Add an equal volume (100 µl) of 96-100% Ethanol to the sample and mix thoroughly by vortexing.** **NOTE**: During RNA purification precipitate may form after the addition of ethanol. This does not affect the procedure.

4. **Apply the sample** (including any precipitate that may have formed) **to a HiBind® RNA spin column placed into a 2ml collection tube** (supplied). **Centrifuge at 10,000 x g for 30 to 60 seconds at room temperature. Discard flow-through and proceed to the next step.**

   NOTE: The maximum capacity of the HiBind® RNA spin column is 800µl Larger volumes can be loaded on to the column successively in the same HiBind® RNA spin column. Discard flow-through after each centrifugation.

5. **Add 500µl of RNA Wash Buffer II diluted with absolute ethanol. Centrifuge at 10,000 x g for 30 to 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.**

6. **Wash the column as before with 500µl of RNA Wash Buffer II diluted with absolute ethanol. Centrifuge at 10,000 x g for 30 to 60 seconds and discard flow-through.**

7. **With the empty 2 ml collection tube, centrifuge the HiBind® RNA column for 2 minutes at maximum speed to completely dry the HiBind® matrix.**

8. **Elution of RNA: Transfer the column into a clean 1.5 ml centrifuge tube** (not supplied), **and elute the RNA with 40-70µl of DEPC-treated water** (supplied). **Make sure that you add the water directly onto the column matrix. Centrifuge for 1 minute at 10,000 x g.** A second elution may be necessary if the expected yield of RNA is > 30 µg. Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase Total RNA yield, the concentration will be lower since more than 80% of RNA has been recovered in the first elution. Pre-heating the water to 70°C before adding it to the column, and incubating the column for 5 min at room temperature before centrifugation may increase yields