cDNA SYNTHESIS

Reverse Transcription (Using SuperScript™ II RT – Invitrogen, Cat. No. 18064-014)

A 20-µL reaction volume can be used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:

   - 1µL 50–250 ng random primers
   - xµL 1 ng to 5 µg total RNA
   - 1µL dNTP Mix (10 mM each)

   Add sterile, distilled water to bring total to 12 µL

2. Heat mixture to 65°C for 5 min and quickly chill on ice. Collect the contents of the tube by brief centrifugation and add (note for >4 samples, prepare a master mix):

   - 4 µL 5X First-Strand Buffer
   - 2 µL 0.1 M DTT
   - 1 µL RNaseOUT™ (40 units/µL) (OPTIONAL)*

   *RNaseOUT™ (Cat. No. 10777-019) is required if using <50 ng starting RNA.

3. Mix contents of the tube gently and incubate at 25°C for 2 min.

4. Add 1 µL (200 units) of SuperScript™ II RT and mix by pipetting gently up and down.

5. Incubations (RT1 program on our machine):

   - 25°C for 10 min.
   - 42°C for 50 min.
   - Inactivate the reaction by heating at 70°C for 15 min.

6. Store at -20°C.