

cDNA SYNTHESIS ACCORDING TO THE KIT

1. Use approximately 1 μ g of RNA and proceeds as follows:
RNA - 1 μ g
Oligo dT vial 5 - 0.5 μ L
Water- variable depending upon RNA volume
Total reaction volume-6.5 μ L
 2. Incubate in thermocycler at 65⁰C for 10 minutes.
 3. Immediately cool on ice/ice water.
 4. Add the following components from the Kit :
Vial 2: 2 μ L
Vial3: 0.25 μ L
Vial 4: 1 μ L
Vial 1: 0.25 μ L
Total added volume is 3.5 μ L. So the final volume will be 10 μ L (6.5 μ L + 3.5 μ L).
 5. Incubate the mix at 50⁰C for 1hr:30min in thermocycler.
 6. Dilute the cDNA with 90 μ L of water to make the final volume of 100 μ L and store in -20⁰C.
- Kit from Roche (Transcriptor First Strand cDNA synthesis kit)