

Reverse Transcription of RNA into cDNA Protocol

Materials:

Components of Thermo Scientific RevertAid First Strand cDNA Synthesis Kit:

- Random Hexamer Primer
- 5 X Reaction Buffer
- RiboLock RNase Inhibitor (20 U/ μ l)
- 10 mM dNTP Mix
- RevertAid M-MiLV RT (200 U/ μ l)
- Water, nuclease free
- 0.2 ml PCR Tubes, pipettes
- 1 μ g of isolated mRNA or 0.1 ng - 5 μ g of total RNA;

First Strand cDNA Synthesis:

1. Add the following reagents into a sterile, nuclease –free tube on ice in the indicated order:
 - 1 μ g Template RNA
 - 1 μ l Random Hexamer Primer
 - to 12 μ l Water, nuclease free

Total volume: 12 μ l
2. If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 10 min. Chill on ice, spin down and place the vial back on ice.
3. Add the following components in the indicated order:
 - 4 μ l 5X Reaction Buffer
 - 1 μ l RiboLock RNase Inhibitor (20 U/ μ l)
 - 2 μ l 10 mM dNTP Mix
 - 1 μ l RevertAid M-MiLV RT (200 U/ μ l)

Total volume: 8 μ l.

Total reaction Mix: 20 μ l.

4. Mix gently and centrifuge briefly.
5. Incubation (in a PCR device):
 - 25 °C 5 min
 - 42 °C 60 min
 - 70 °C 5 min

The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one week. For longer storage: -70°C is recommended.