cDNA Synthesis for RT-PCR Protocol

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National Institutes of Health

Reagents

5X First Strand Buffer
Invitrogen, Cat. Y00146
10mM dNTP Set
Amersham Biosciences, Cat. 27-2035-01
0.1M DTT
Invitrogen, Cat. Y00147
Random Primers
Invitrogen, Cat. 48190-011
RNaseOUT Ribonuclease Inhibitor
Invitrogen, Cat. 10777-019
SuperScript II RNase H- Reverse Transcriptase
Invitrogen, Cat. 18064-014

Procedure

1. Combine 3-5 μg total RNA and molecular grade water to 8 μl final volume.
2. Add 3 μl Random Primers.
3. Add 1 μl dNTP mix.
4. Vortex and then spin down tube.
5. Incubate at 65°C for 5 min.
6. Place tube on ice.
7. Add 4 μl 5X Buffer, 2 μl DTT and 1μl RNAseOut.
8. Vortex and then spin down tube.
9. Incubate at 42°C for 1 min.
10. Add 1μl SuperScript II RNase H- Reverse Transcriptase.
11. Incubate at 42°C for 60 min.
12. Incubate at 70°C for 15 min.
13. Add 180 μl molecular grade water.

14. Use Nanodrop 1000 to measure concentration. Set sample type setting to Other Sample and the constant to 33.

15. Store at -80°C.

NOTES

1. 10 mM dNTP Mix:
   10 µl dATP [100mM]
   10 µl dCTP [100mM]
   10 µl dGTP [100mM]
   10 µl dTTP [100mM]
   60 µl molecular grade water