MCLAB
Molecular Cloning Laboratories

User Manual

Version 5.1

Product name: QuantumScript™ Reverse Transcriptase

Cat #: SSII-25, SSII-50, SSII-100, SSII-200, SSII-OEM, B-RB5

Description:

QuantumScript™ Reverse Transcriptase is a newly engineered version of M-MLV reverse transcriptase with a minimum RNase H activity and enhaced thermostability. The enzyme is purified to homogeneity to ensure the best performance. The optimal fist-strand cDNA synthesis temperature for this enzyme is 42˚C, with cDNA product size from 100 bp to 7 Kb.

Protocol:

The following procedure uses 10 pg to 5 µg of total RNA or 10 pg to 500 ng of mRNA.

1. In a sterile RNase-free microcentrifuge tube, add primers (200-500 ng of oligo $(dT)_{12-18}$, 50-250 ng of random primer or 2 pmol of specific primers). Heat the tube to 70°C for 5 minutes and incubate on ice for 1 min to denature any possible secondary structure within the template. Spin briefly to collect the solution at the bottom of the tube.

2. Add the following components to the annealed primer/template in the order below:

   Note: Do not alter the ratio of primer to mRNA.
   5 µl 5X Reaction Buffer
   1 µl of 10mM dNTP mixture (10mM each dATP, dGTP, dCTP and dTTP)
   0.5 µl QuantumScript Reverse Transcriptase (100 u/ µl)
   1ul DTT (20uM)
   1ul RNAse inhibitor
   Add nuclease-free water to a final volume of 25µl

3. Mix gently. For random primers, incubate the tube at 25°C for 5 min. Perform first-strand synthesis at 42° C for 30-60 min. Reaction temperature may be optimized between 50°C-60°C for difficult template with high secondary structures.

4. Inactivate the enzyme by incubation at 70°C for 15 min after the reaction.

5. When perform PCR amplification after step 4, removal of RNA is highly recommended prior to the PCR amplification to ensure the yield of PCR product. Addition of 2 units of RNase H (Cat. # RNHE-100, RNHE-200, RNHE-300) and 20 min incubation at 37 °C is recommended for the removal of RNA. Standard protocols for second-strand synthesis can be found in reference 2.

   Note: The 5X Reaction Buffer is compatible with enzymes used in a number of downstream applications. Typically there is no need for phenol extractions or ethanol precipitations using this protocol before any PCR amplification.

References:

1. Roth, M.J., Tanese, N. and Goff, S.P. (1985) Purification and characterization of murine retroviral reverse transcriptase expressed in Escherichia coli. J. Biol. Chem. 260, 9326–35.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: Molecular Cloning: A; Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.