MCLAB
Molecular Cloning Laboratories

User Manual

Version 6.1

Product name: QuantumScript™ HD Reverse Transcriptase

Cat #: SSIII-50, SSIII-100, SSIII-200, SSIII-300, SSIII-OEM, B-RB5

Description:

QuantumScript™ HD Reverse Transcriptase is a newly engineered version of QuantumScript™ Reverse Transcriptase with increased sensitivity, improved specificity and maximum thermostability. QuantumScript™ HD Reverse Transcriptase has been engineered to have a longer half life at 50°C, which enables its ability to process longer RNA with more complexed secondary structures. The enhanced thermostability of this enzyme is obtained through a re-engineered RNA-based DNA Polymerase domain and a fusion of a novel RNA-interacting surface domain at the RNase H domain site. The enzyme is purified to homogeneity to ensure its best performance. The optimal fist-strand cDNA synthesis temperature for this enzyme is 50°C, and it has a broad working temperature range from 37°C to 55°C, with cDNA product size from 100 bp to 12 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 primers 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 structures 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)
   25 units RNAse Inhibitor
   0.5 µl QuantumScript Reverse Transcriptase (200 u/µl)
   1ul DTT (20uM)
   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 55°C for 30-60 min. Reaction temperature may be optimized between 50°C-60°C for difficult template with high secondary structure.
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.

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.