BioChain®
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Certificate of Analysis

Product Name: UltraScript™ Reverse Transcriptase

UltraScript™ Reverse Transcriptase is a new engineered version of M-MLV reverse transcriptase with minimum RNase H activity and maximum thermal stability. The enzyme is purified to near homogeneity to ensure high thermal stability, high specificity, high fidelity, high yield and more full length cDNA synthesis that the premium reverse transcriptase provides. The optimal fist-strand cDNA synthesis temperature for this enzyme is 55°C, and it has a broad working temperature range from 37°C to 60°C, with cDNA product size from 100 bp to 12 Kb.

Catalog No.	**L4121100 and L4121050**
Lot No.	**C105179**
Source	*E.coli*
Size	100,000 units or 50,000 units with 3 ml or 1.5 ml 5x reaction buffer, respectively
Concentration	200 u/µl
Storage Buffer	20 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.05% (v/v)Triton X-100, 0.1 mM EDTA, 0.1 M NaCl and 50% (v/v) glycerol.
Reaction Buffer (5x)	250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$, and 50 mM DTT
Unit Definition	One unit of the enzyme incorporates I nmole of dTTP into acid-precipitable material in 10 minutes at 37˚C using poly (A): oligo $(dT)_{25}$ as template-primer.
Quality Control	This enzyme has passed the quality control assays: SDS-PAGE analysis for purity, functional absence of endonuclease/nickase activities, functional absence of exonuclease activities, functional absence of protease activity.

Storage and Handling -20˚C

Protocol

First-Strand cDNA Synthesis

Materials to Be Supplied by the User

- Recombinant RNasin Ribonuclease Inhibitor (Cat.# Z5040001)
- dATP, 10mM (Cat.# K6011101-400 , 100mM)
- dCTP, 10mM (Cat.# K6011103-400 , 100mM)
- dGTP, 10mM (Cat.# K6011102-400 , 100mM)
- dTTP, 10mM (Cat.# K6011104-400 , 100mM)
- Nuclease-Free Water

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 shown.
Note: Do not alter the ratio of primer to mRNA.
5 µl 5X Reaction Buffer; 5 µl of 10mM dNTP mixture (10mM each dATP, dGTP, dCTP and dTTP)
25 units Recombinant RNasin Ribonuclease Inhibitor
1 µl UltraScript Reverse Transcriptase (200 u/µl)
Add nuclease-free water to final volume 25µl

3. Mix gently. For random primers, incubate 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 reaction.

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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. # Z5040006) and 20 min incubation at 37 ^{o}C is recommended for the removal of RNA. Standard PCR protocols for second-strand synthesis may 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.

IV. 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.

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