SUPERSCRIPT™ II
RNase H⁻ Reverse Transcriptase

Cat. No. 18064-014
Lot No. _____ 10,000 units; 200 U/µl

Exp. Date: ____. Store at -20°C (not frost-free).

SUPERSCRIPT II RNase H⁻ Reverse Transcriptase (U.S. Patent 5,244,797) is purified to near homogeneity from *E. coli* containing the *pol* gene of Moloney Murine Leukemia Virus (1,2). The enzyme is used to synthesize first strand cDNA and will generally give higher yields of cDNA and more full length product than other reverse transcriptases.

Components:

18064-014	SUPERSCRIPT II	Lot No.
Y00146	5X First Strand Buffer	Lot No.
Y00147	0.1 M DTT	Lot No.

Unit Definition:
One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo$(dT)_{25}$ as template-primer (3).

Storage Buffer:
20 mM Tris-HCl (pH 7.5)
100 mM NaCl
0.1 mM EDTA
1 mM DTT
0.01% (v/v) NP-40
50% (v/v) glycerol

Additional Components Provided:
The 5X First Strand Buffer [250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$] and 0.1 M DTT are provided. Store both solutions at -20°C. Thaw the solutions at room temperature just prior to use and refreeze immediately.

Doc. Rev.: 101397

This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Life Technologies TECH-LINE℠ (800) 828-6686].

Quality Control Assays:

This product has passed the following quality control assays:
SDS-polyacrylamide gel analysis for purity; functional absence of endodeoxyribonuclease, 3′ and 5′ exodeoxyribonuclease, and ribonuclease activities; yield and length of cDNA product.

Note: Limited Label License

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First Strand cDNA Synthesis Using SUPERSCRIPT II for RT-PCR:

A 20-µl reaction volume can be used for 1-5 µg of total RNA or 50-500 ng of mRNA. Add the following components to a nuclease-free microcentrifuge tube:

1 µl Oligo $(dT)_{12\text{-}18}$ (500 µg/ml)[a]
1-5 µg total RNA
Sterile, distilled water to 12 µl

Heat mixture to 70°C for 10 min and quick chill on ice. Collect the contents of the tube by brief centrifugation and add:

4 µl 5X First Strand Buffer
2 µl 0.1 M DTT
1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)

Mix contents of the tube gently and incubate at 42°C for 2 min. Add 1 µl (200 units) of SUPERSCRIPT II, mix by pipetting gently up and down. Incubate 50 min at 42°C. Inactivate the reaction by heating at 70°C for 15 min. The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (those > 1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µl (2 units) of *E. coli* RNase H and incubate 37°C for 20 min.

[a] Alternatively 50-250 ng of random primers or 2 pmole of a gene specific primer may be used. Use of random primers requires incubation at 25°C for 10 min before the 42°C incubation.

PCR Reaction

Use only 10% of the first strand reaction for PCR. Adding larger amounts of he first strand reaction may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR reaction tube for a final reaction volume of 100 µl:

10 µl	10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]
3 µl	50 mM $MgCl_2$[b]
2 µl	10 mM dNTP Mix
1 µl	amplification primer 1 (10 µM)
1 µl	amplification primer 2 (10 µM)
1 µl	*Taq* DNA polymerase (5 U/µl)
2 µl	cDNA (from first strand reaction)
80 µl	autoclaved, distilled water

2. Mix gently and layer 2 drops (~100 µl) of silicone oil over the reaction. *(Note: the addition of silicone oil is unnecessary in thermal cyclers equipped with a heated lid.)*
3. Heat reaction to 94°C for 3 min to denature.
4. Perform 15 to 40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.

[b] Optimal concentration of $MgCl_2$ needs to be determined empirically for each template primer pair.

References:

1. Kotewicz, M.L., D'Alessio, J.M., Driftmier, K.M., Blodgett, K.P., and Gerard, G.F. (1985) *Gene 35*, 249.
2. Gerard, G.F., D'Alessio, J.M., Kotewicz, M.L., and Noon, M.C. (1986) *DNA 5*, 271.
3. Houts, G.E., Miyagi, M., Ellis, C., Beard, A., and Beard, J.W. (1979) *J. Virol. 29*, 517.