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SuperScript™ III Reverse Transcriptase

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Cat. No. 18080-093 Buy Now **Size: 2,000 units**
18080-044 Buy Now **10,000 units**
18080-085 Buy Now **4 × 10,000-unit kit**

Conc: 200 U/µl **Store at -20°C (non-frost-free)**

Description

SuperScript™ III Reverse Transcriptase is an engineered version of M-MLV RT with reduced RNase H activity and increased thermal stability. The enzyme is purified to near homogeneity from *E. coli* containing the modified *pol* gene of Moloney Murine Leukemia Virus (1,2). The enzyme can be used to synthesize first-strand cDNA at temperatures up to 55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. It can generate cDNA from 100 bp to >12 kb.

Component	2,000 U Kit	10,000 U Kit
SuperScript™ III RT (200 U/µl)	10 µl	50 µl
5X First-Strand Buffer*	1000 µl	1000 µl
0.1 M DTT	500 µl	500 µl

*[250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$]

Unit Definition

One unit incorporates 1 nmol 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

Storage

Store all components at -20°C (non-frost-free). Thaw 5X First-Strand Buffer and 0.1 M DTT at room temperature just prior to use and refreeze immediately.

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First-Strand cDNA Synthesis

The following 20-µl reaction volume can be used for 10 pg–5 µg of total RNA or 10 pg–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:
   1 µl of oligo(dT)$_{20}$ (50 µM); *or* 200–500 ng of oligo(dT)$_{12\text{-}18}$; *or* 50–250 ng of random primers; *or* 2 pmol of gene-specific primer
   10 pg–5 µg total RNA *or* 10 pg–500 ng mRNA
   1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)
   Sterile, distilled water to 13 µl
2. Heat mixture to 65°C for 5 minutes and incubate on ice for at least 1 minute
3. Collect the contents of the tube by brief centrifugation and add:
   4 µl 5X First-Strand Buffer
   1 µl 0.1 M DTT
   1 µl RNaseOUT™ Recombinant RNase Inhibitor (Cat. no. 10777-019, 40 units/µl). Note: When using less than 50 ng of starting RNA, the addition of RNaseOUT™ is essential.
   1 µl of SuperScript™ III RT (200 units/µl)*
   *If generating cDNA longer than 5 kb at temperatures above 50°C using a gene-specific primer or oligo(dT)$_{20}$, the amount of SuperScript™ III RT may be raised to 400 U (2 µl) to increase yield.
4. Mix by pipetting gently up and down. If using random primers, incubate tube at 25°C for 5 minutes.
5. Incubate at 50°C for 30–60 minutes. Increase the reaction temperature to 55°C for gene-specific primer. Reaction temperature may also be increased to 55°C for difficult templates or templates with high secondary structure.
6. Inactivate the reaction by heating at 70°C for 15 minutes.

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 at 37°C for 20 minutes.

PCR Reaction

The following example reaction is recommended as a starting point:

1. Add the following to a PCR reaction tube:

10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5 µl
50 mM $MgCl_2$*	1.5 µl
10 mM dNTP Mix	1 µl
Sense primer (10 µM)	1 µl
Antisense primer (10 µM)	1 µl
Taq DNA polymerase (5 U/µl)	0.4 µl
cDNA (from first-strand reaction)	2 µl
Autoclaved, distilled water	to 50 µl

2. Mix gently and layer 1–2 drops (~50 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 2 minutes to denature.
4. Perform 15–40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.

*Optimal concentration of $MgCl_2$ needs to be determined empirically for each template-primer pair.

Quality Control

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.

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.

Related Products

	Quantity	Cat. No.
Oligo(dT)$_{20}$ Primer (50 µM)	50 µl	18418-020
Oligo(dT)$_{12\text{-}18}$ Primer	25 µg	18418-012
Random Primers	A_{260} units	48190-011
Custom Gene-Specific Primers	visit www.invitrogen.com/oligos	
10 mM dNTP Mix	100 µl	18427-013
DEPC-treated Water	4 × 1.25 ml	10813-012
RNAseOUT™ Recombinant Ribonuclease Inhibitor (40 U/µl)	5,000 units	10777-019
RNase H	30 units	18021-014
Platinum® *Taq* DNA Polymerase	100 units	10966-018