invitrogen™

SuperScript™ III Reverse Transcriptase

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

Conc: 200 U/ l **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/ l)	10 l	50 l
5X First-Strand Buffer*	1000 l	1000 l
0.1 M DTT	500 l	500 l

*[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- l reaction volume can be used for 10 pg–5 g of total RNA or 10 pg–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:
   1 l of oligo(dT)$_{20}$ (50 M); *or* 200–500 ng of oligo(dT)$_{12-18}$; *or* 50–250 ng of random primers; *or* 2 pmol of gene-specific primer
   10 pg–5 g total RNA *or* 10 pg–500 ng mRNA
   1 l 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH)
   Sterile, distilled water to 13 l
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 l 5X First-Strand Buffer
   1 l 0.1 M DTT
   1 l RNaseOUT™ Recombinant RNase Inhibitor (Cat. no. 10777-019, 40 units/ l). Note: When using less than 50 ng of starting RNA, the addition of RNaseOUT™ is essential.
   1 l of SuperScript™ III RT (200 units/ l)*
   *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 l) 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 l (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 l
50 mM $MgCl_2$*	1.5 l
10 mM dNTP Mix	1 l
Sense primer (10 M)	1 l
Antisense primer (10 M)	1 l
Taq DNA polymerase (5 U/ l)	0.4 l
cDNA (from first-strand reaction)	2 l
Autoclaved, distilled water	to 50 l

2. Mix gently and layer 1–2 drops (~50 l) 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 M)	50 l	18418-020
Oligo(dT)$_{12-18}$ Primer	25 g	18418-012
Random Primers	A_{260} units	48190-011
Custom Gene-Specific Primers	visit www.invitrogen.com/oligos	
10 mM dNTP Mix	100 l	18427-013
DEPC-treated Water	4 × 1.25 ml	10813-012
RNAseOUT™ Recombinant Ribonuclease Inhibitor (40 U/ l)	5,000 units	10777-019
RNase H	30 units	18021-014
Platinum® *Taq* DNA Polymerase	100 units	10966-018

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