

SuperScript™ III Reverse Transcriptase

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Cat. No. 18080-093
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Size: 2,000 units
18080-044
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10,000 units
18080-085
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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

SuperScript™ III RT (200 U/μl)

2,000 U Kit

10 μl

10,000 U Kit

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 MgCl₂]

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.

Part no. 18080.pps

Rev. date: 7 Dec 2004

First-Strand cDNA Synthesis

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

1. Add the following components to a nuclease-free microcentrifuge tube:
1 μ l of oligo(dT)₂₀ (50 μ 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 μ g total RNA *or* 10 pg–500 ng mRNA
1 μ l 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at
neutral pH)
Sterile, distilled water to 13 μ 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 μ l 5X First-Strand Buffer

1 μ l 0.1 M DTT

1 μ l RNaseOUT™ Recombinant RNase Inhibitor (Cat. no. 10777-019,
40 units/ μ l). Note: When using less than 50 ng of starting RNA, the
addition of RNaseOUT™ is essential.

1 μ l SuperScript™ III RT (200 units/ μ 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 μ 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 μ 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:

- Add the following to a PCR reaction tube:

10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5 μ l
50 mM MgCl ₂ *	1.5 μ l
10 mM dNTP Mix	1 μ l
Sense primer (10 μ M)	1 μ l
Antisense primer (10 μ M)	1 μ l
<i>Taq</i> DNA polymerase (5 U/ μ l)	0.4 μ l
cDNA (from first-strand reaction)	2 μ l
Autoclaved, distilled water	to 50 μ l
- Mix gently and layer 1–2 drops (~50 μ l) of silicone oil over the reaction.
(*Note: The addition of silicone oil is unnecessary in thermal cyclers equipped with a heated lid.*)
- Heat reaction to 94°C for 2 minutes to denature.
- Perform 15–40 cycles of PCR. Annealing and extension conditions are primer and template dependent and must be determined empirically.
*Optimal concentration of MgCl₂ 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

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

Related Products

	<u>Quantity</u>	<u>Cat. No.</u>
Oligo(dT) ₂₀ Primer (50 µM)	50 µl	18418-020
Oligo(dT) ₁₂₋₁₈ 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® <i>Taq</i> DNA Polymerase	100 units	10966-018

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