

SuperScript™ III Reverse Transcriptase

Cat. No. **18080-093**
18080-044
18080-085

Size: **2,000 units**
10,000 units
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.

<u>Component</u>	<u>2,000 U Kit</u>	<u>10,000 U Kit</u>
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 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 of 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

Limited Use Label License No. 4: Products for PCR which do not include any rights to perform PCR

This product is optimized for use in the Polymerase Chain Reaction (PCR) covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche, Ltd. ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as Invitrogen, when used in conjunction with an Authorized Thermal Cycler, or is available from Applied Biosystems. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Limited Use Label License No. 138: SuperScript™ III Reverse Transcriptase

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any commercial purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for commercial purposes. Commercial purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

©2002–04 Invitrogen Corporation. All rights reserved.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.