SuperScript™ II Reverse Transcriptase

Cat. No. 18064-022 **Size: 2,000 units**
Cat. No. 18064-014 **Size: 10,000 units**
Cat. No. 18064-071 **Size: 4 × 10,000 units**

Conc. 200 U/µL **Store at –20°C (non-frost-free)**

Description

SuperScript™ II Reverse Transcriptase (RT) 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 higher temperatures than conventional M-MLV RT, providing increased specificity, higher yields of cDNA, and more full-length product. It can generate cDNA up to 12.3 kb.

Components

SuperScript™ II RT, 5X First-Strand Buffer (250 mM Tris-HCl, pH 8.3 at room temperature; 375 mM KCl; 15 mM $MgCl_2$), 0.1 M DTT

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 Conditions

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

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).

Intended Use

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

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First-Strand cDNA Synthesis Using SuperScript™ II RT

A 20-µL reaction volume can be used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA.

1. Add the following components to a nuclease-free microcentrifuge tube:

Oligo(dT)$_{12-18}$ (500 µg/mL) *or* 50–250 ng random primers *or* 2 pmole gene-specific primer (GSP)	1 µL
1 ng to 5 µg total RNA *or* 1–500 ng of mRNA	x µL
1 µL dNTP Mix (10 mM each)	1 µL
Sterile, distilled water	to 12 µL

2. Heat mixture to 65°C for 5 min and quick chill on ice. Collect the contents of the tube by brief centrifugation and add:

5X First-Strand Buffer	4 µL
0.1 M DTT	2 µL
RNaseOUT™ (40 units/µL) (optional)*	1 µL

*RNaseOUT™ (Cat. No. 10777-019) is required if using <50 ng starting RNA.

3. Mix contents of the tube gently. If you are using oligo(dT)$_{12-18}$ or GSP, incubate at 42°C for 2 min. If you are using random primers, incubate at 25°C for 2 min.
4. Add 1 µL (200 units) of SuperScript™ II RT and mix by pipetting gently up and down.
   If you are using less than 1 ng of RNA, reduce the amount of SuperScript™ II RT to 0.25 µL (50 units) and add sterile, distilled water to a 20 µL final volume.

   If you are using random primers, incubate tube at 25°C for 10 min.
5. Incubate at 42°C for 50 min.
6. Inactivate the reaction by heating at 70°C for 15 min.

First-Strand cDNA Synthesis Using SuperScript™ II RT, Continued

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (>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 min.

PCR

The following is intended as a guideline and starting point when using first-strand cDNA in PCR with *Taq* DNA polymerase. The optimal concentration of Mg^{++} will vary depending on the template and primer pair.

Use only 10% of the first-strand reaction for PCR. Higher volumes may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR 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
Forward primer (10 µM)	1 µL
Reverse 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 with 1–2 drops (~50 µL) of silicone oil. *(Note: silicone oil is unnecessary in thermal cyclers equipped with a heated lid.)*
3. Heat reaction to 94°C for 2 min to denature.
4. Perform 15 to 40 cycles of PCR. Use the recommended annealing and extension conditions for your *Taq* DNA polymerase.

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available at www.invitrogen.com/support.

Additional Products

RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µL) is available separately from Invitrogen (Cat. no. 10777-019).

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.
4. Kotewicz, M.L., Sampson, C.M., D'Alessio, J.M., and Gerard, G.F. (1988) *Nuc. Acids Res. 16*, 265.

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