Invitrogen™ Lyo-ready SuperScript™ III Reverse Transcriptase

Pub. No. MAN0016720 Rev. B.0

invitrogen

Print options

Package contents

Sample Kit No.	Concentration	Size
EP184B2SMP2	600 U/µL	18,000 U

Kit contents

Storage conditions

Store at –20°C (non-frost-free).
Product is designed to withstand at least 10 freeze-thaw cycles.

Required materials

- Template: RNA
- Oligo(dT)$_{20}$ primer (Cat. No. 18418020), Random Hexamers (Cat. No. N8080127), or gene-specific primers
- RNAseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019)
- *E. coli* Ribonuclease H (RNase H) (Cat. No. 18021014)
- UltraPure™ DEPC-treated water (Cat. No. 10813012)

Timing

- Preparation time: 10 minutes
- Run time: <1.5 hour

Product description

- Invitrogen™ Lyo-ready SuperScript™ III Reverse Transcriptase (RT) is an engineered version of M-MuLV Reverse Transcriptase with reduced RNase H activity and increased thermal stability. The lyo-ready enzyme formulation combines feasibility to lyophilize with favorable enzyme performance properties of the standard enzyme version with glycerol.
- 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.

Online resources

For further information, contact LCSVilnius@thermofisher.com.

Protocol outline

A. Anneal primer to RNA
B. Assemble reaction mix
C. Add reaction mix to annealed RNA

RT reaction setup

Component	20-µL rxn	Custom	Final conc.
Template RNA[1]	varies	µL	varies
50 µM Oligo d(T)$_{20}$ primer, or 50 µM random hexamers, or 2 µM gene-specific primer	1 µL 1 µL 1 µL	µL	2.5 µM 2.5 µM 0.1 µM
5X First-Strand Buffer	4 µL	µL	1X
10 mM dNTP mix (10 mM each)	1 µL	µL	0.5 mM each
0.1 M DTT	1 µL	µL	5 mM
RNAseOUT™ RNase Inhibitor (40 U/µL)	1 µL	µL	2 U/µL
Lyo-ready SuperScript™ III RT (200 U/µL)[2]	1 µL	µL	10 U/µL
DEPC-treated water	to 20 µL	to µL	N/A

[1] 10 pg–1 µg total RNA or 10 pg–500 ng mRNA.
[2] Prior to use, dilute lyo-ready SuperScript™ III RT to 200 U/µL with lyo-ready RT Diluent included in the kit. Diluted enzyme can be stored at 4°C for up to one week.

RT protocol

Go to page 2 for instructions on preparing and running your RT experiment.

Limited Warranty, Disclaimer, and Licensing Information

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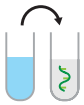
First-strand cDNA synthesis reaction

The example procedure below shows the appropriate volumes for a single **20-µL** two-step RT-PCR. For multiple reactions, prepare a master mix of components to minimize pipetting error, then dispense the appropriate volumes into each reaction tube before adding annealed template RNA and primers.

<table>
<tr><th>Steps</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Anneal primer to template RNA</td><td>
a. Mix and briefly centrifuge all reaction components after thawing and keep on ice.
b. Combine the following components in a sterile, nuclease-free tube on ice in the indicated order.
Note: Consider the volumes for all components listed in steps 1 and 2 to determine the correct amount of water required to reach your final reaction volume.
<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>50 µM Oligo d(T)$_{20}$ primer, 50 µM random hexamers, or
2 µM gene-specific primer</td><td>1 µL</td></tr>
<tr><td>10 mM dNTP mix (10 mM each)</td><td>1 µL</td></tr>
<tr><td>Template RNA[1]</td><td>up to 11 µL</td></tr>
<tr><td>DEPC-treated water</td><td>to 13 µL</td></tr>
</table>
[1] 10 pg–1 µg total RNA or 10 pg–500 ng mRNA.
c. Mix and briefly centrifuge the components.
d. Heat the RNA-primer mix at 65°C for 5 minutes, and then incubate on ice for at least 1 minute, centrifuge briefly, and place on ice.
</td></tr>
<tr><td>2  </td><td>Prepare RT reaction mix</td><td>
a. Combine the following components in the indicated order in a sterile, nuclease-free tube on ice.
<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>5X First-Strand Buffer</td><td>4 µL</td></tr>
<tr><td>0.1 M DTT</td><td>1 µL</td></tr>
<tr><td>RNaseOUT™ RNase Inhibitor (40 U/µL)</td><td>1 µL</td></tr>
<tr><td>Lyo-ready SuperScript™ III RT (200 U/µL)[1]</td><td>1 µL</td></tr>
</table>
[1] Prior to use, dilute lyo-ready SuperScript™ III RT to 200 U/µL with lyo-ready RT Diluent included in the kit.
b. Mix gently and then briefly centrifuge the components.
</td></tr>
<tr><td>3  </td><td>Combine annealed RNA and RT reaction mix</td><td>Add RT reaction mix to the annealed RNA.</td></tr>
</table>

Steps	Action	Procedure details
4 	**Incubate reactions**	**a.** If using random hexamer, incubate the combined reaction mixture at 25°C for 5 minutes, and then proceed to step b. If using oligo $d(T)_{20}$ or gene-specific primer, directly proceed to step b. **b.** If using random hexamer or oligo $d(T)_{20}$ or gene-specific primer, incubate the combined reaction mixture at 50°C for 30–60 minutes. If using gene-specific primer, incubate the combined reaction mixture at 55°C for 30–60 minutes. **Note:** You can also increase the reaction temperature to 55°C for difficult templates or templates with high secondary structure. **c.** Inactivate the reaction by incubating it at 70°C for 15 minutes.
5 	***Optional*: Remove RNA**	To remove RNA, add 1 µL of *E. coli* RNase H, and incubate 37°C for 20 minutes. **Note:** Amplification of some PCR targets (>1 kb) may require removal of RNA complementary to the cDNA.
6 	**PCR amplification**	Use the RT reaction product directly in PCR or store it at –20°C. Use 2 µL of the RT reaction mix in 50 µL of final PCR volume. **Note:** As a recommended starting point for PCR, the reverse transcription reaction (cDNA) volume should be 2–5 µL.