SuperScript™ IV First-Strand Synthesis System

USER GUIDE Pub. No. MAN0013442 Rev. C.0

invitrogen

Print Options

Package contents

Catalog Number	Size
18091050, 18091150	50 reactions
18091200, 18091300	200 reactions

Kit Contents

Storage conditions

Store at –20°C (non-frost-free)

Required materials

- Template: RNA
- *(Optional)* 2 µM gene-specific primers

Timing

- Preparation time: 10 minutes
- Run time: 20 minutes

Selection guides

Go online to learn more.

PCR Enzymes and Master Mixes
RT Enzymes and Kits
Real-Time PCR Instruments
Real-Time PCR Master Mixes
PCR Thermal Cyclers

Product description

For first strand cDNA synthesis using total RNA or poly(A)+-selected RNA primed with oligo(dT), random primers, or a gene-specific primer.

Important guidelines

Pre-warm the 5× SSIV Buffer to room temperature before use. Vortex and briefly centrifuge the buffer prior to preparing the reverse transcription reaction mix.

Online resources

Visit our product page for additional information and protocols.
For support, visit thermofisher.com/support.

Protocol outline

A. *(Optional)* Remove genomic DNA
B. Anneal primers to RNA
C. Assemble reaction mix
D. Add reaction mix to annealed RNA

RT reaction setup

Use the measurements below to prepare your RT reaction, or enter your own parameters in the column provided.

Component	20-µL rxn	Custom	Final Conc.
DEPC-treated water	to 20 µL	to µL	N/A
5× SSIV Buffer	4.0 µL	µL	1×
10 mM dNTP mix (10 mM each)	1.0 µL	µL	0.5 mM each
100 mM DTT	1.0 µL	µL	5 mM
Ribonuclease Inhibitor (40 U/µL)	1.0 µL	µL	2.0 U/µL
50 µM Oligo $d(T)_{20}$ primer, or 50 ng/µL random hexamers, or 2 µM gene-specific primer	1.0 µL 1.0 µL 1.0 µL	µL	2.5 µM 2.5 ng/µL 0.1 µM
Template RNA*	varies	µL	< 5 µg total RNA or < 500 ng mRNA

* 10 pg–5 µg total RNA or 10 pg–500 ng mRNA

RT protocol

Go to page 3 for instructions on preparing and running your RT experiment.

Optimization strategies and troubleshooting

Refer to the pop-ups below for guidelines to optimize and troubleshoot your RT reaction.

RNA Sample Prep
RT Guidelines
Troubleshooting

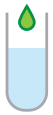
Limited Warranty, Disclaimer, and Licensing Information

ThermoFisher SCIENTIFIC

Optional: Remove genomic DNA

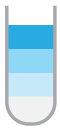
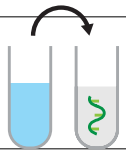
The following example procedure describes the required to remove the genomic DNA (gDNA) from your samples for the RT and No RT Control reactions using the ezDNase™ Enzyme (supplied with Cat. No. 18091150 and 18091300; also available separately at **thermofisher.com**).

The volumes given are for a single **10-µL** gDNA digestion reaction . For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into each reaction.

<table>
<tr><th colspan="2">Step</th><th>Action</th></tr>
<tr><td>1</td><td>  </td><td>Prepare gDNA digestion reaction mix (on ice)</td><td>For each RT reaction and No RT Control reaction, prepare a 10-µL gDNA reaction mix in an RNase-free tube on ice with the following components:
<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>10× ezDNase™ Buffer</td><td>1 µL</td></tr>
<tr><td>ezDNase™ Enzyme</td><td>1 µL</td></tr>
<tr><td>Template RNA (1 pg to 2.5 µg total RNA)</td><td>up to 8 µL</td></tr>
<tr><td>Nuclease-free water</td><td>to 10 µL</td></tr>
</table></td></tr>
<tr><td>2</td><td>  </td><td>Digest gDNA</td><td>Gently mix and incubate at 37°C for 2 minutes.</td></tr>
<tr><td>3</td><td>  </td><td>Inactivate ezDNase™ enzyme</td><td>If the RNA sample is to be used for RT-PCR of fragments ≥3 kb, incubate the sample for 5 minutes at 55°C in the presence of 10 mM DTT to inactivate the enzyme.</td></tr>
<tr><td>4</td><td>  </td><td>Place the reaction on ice</td><td>Chill the tube on ice to bring the sample to room temperature, then briefly centrifuge and place the tube on ice.</td></tr>
</table>

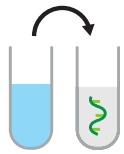
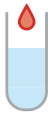
SuperScript™ IV First-Strand cDNA Synthesis Reaction

The following example procedure shows appropriate volumes for a single **20-µL** reverse transcription reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into each reaction tube prior to adding annealed template RNA and primers.

<table>
<tr><th colspan="2">Step</th><th>Action</th></tr>
<tr><td>1</td><td>  </td><td>Anneal primer to template RNA</td><td>a. Combine the following components in a PCR reaction tube.
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 ng/µL random hexamers, or 2 µM gene-specific reverse 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 (10 pg–5 µg total RNA or 10 pg–500 ng mRNA)</td><td>up to 11 µL</td></tr><tr><td>DEPC-treated water</td><td>to 13 µL</td></tr></table>b. Mix and briefly centrifuge the components.
c. Heat the RNA-primer mix at 65°C for 5 minutes, and then incubate on ice for at least 1 minute.</td></tr>
<tr><td>2</td><td>  </td><td>Prepare RT reaction mix</td><td>a. Vortex and briefly centrifuge the 5× SSIV Buffer.
b. Combine the following components in a reaction tube.
<table><tr><th>Component</th><th>Volume</th></tr><tr><td>5× SSIV Buffer</td><td>4 µL</td></tr><tr><td>100 mM DTT</td><td>1 µL</td></tr><tr><td>Ribonuclease Inhibitor</td><td>1 µL</td></tr><tr><td>SuperScript™ IV Reverse Transcriptase (200 U/µL)</td><td>1 µL</td></tr></table>c. Cap the tube, mix, and then briefly centrifuge the contents.</td></tr>
<tr><td>3</td><td>  </td><td>Combine annealed RNA and RT reaction mix</td><td>Add RT reaction mix to the annealed RNA.</td></tr>
<tr><td>4</td><td>  </td><td>Incubate reactions</td><td>a. If using random hexamer, incubate the combined reaction mixture at 23°C for 10 minutes, and then proceed to step b.
If using oligo d(T)$_{20}$ or gene-specific primer, directly proceed to step b.
b. Incubate the combined reaction mixture at 50–55°C for 10 minutes.
c. Inactivate the reaction by incubating it at 80°C for 10 minutes.</td></tr>
<tr><td>5</td><td>  </td><td>(Optional) Remove RNA</td><td>Note: Amplification of some PCR targets (>1 kb) may require removal of RNA.
To remove RNA, add 1 µL E. coli RNase H, and incubate 37°C for 20 minutes.</td></tr>
<tr><td>6</td><td>  </td><td>PCR amplification</td><td>Use your RT reaction immediately for PCR amplification or store it at –20°C.
Note: As a recommended starting point for PCR, reverse transcription reaction (cDNA) should compose 10% of the total reaction volume</td></tr>
</table>

SuperScript™ IV Control Reactions - cDNA synthesis reaction

Follow the procedure below to perform the cDNA synthesis step of the SuperScript™ IV RT-PCR control reactions.

<table>
<tr><th colspan="2">Step</th><th>Action</th></tr>
<tr><td>1</td><td>  </td><td>Anneal primer to template RNA</td><td>a. Prepare two tubes for annealing primer to template RNA. In each tube, combine the following:
<table><tr><th>Component</th><th>Volume</th></tr><tr><td>50 µM Oligo d(T)$_{20}$ primer</td><td>1 µL</td></tr><tr><td>10 mM dNTP mix (10 mM each)</td><td>1 µL</td></tr><tr><td>10 ng/µL total HeLa RNA (10 ng total)</td><td>1 µL</td></tr><tr><td>DEPC-treated water</td><td>10 µL</td></tr></table>b. Mix and briefly centrifuge the components.
c. Heat the RNA-primer mix at 65°C for 5 minutes, and then incubate on ice for at least 1 minute.</td></tr>
<tr><td>2</td><td>  </td><td>Prepare RT reaction mix</td><td>a. Vortex and briefly centrifuge the 5× SSIV Buffer.
b. Prepare two reactions. In each reaction tube, combine the following:
<table><tr><th>Component</th><th>Volume</th></tr><tr><td>5× SSIV Buffer</td><td>4 µL</td></tr><tr><td>100 mM DTT</td><td>1 µL</td></tr><tr><td>Ribonuclease Inhibitor</td><td>1 µL</td></tr><tr><td>SuperScript™ IV Reverse Transcriptase (positive control) or DEPC-treated water (no RT control)</td><td>1 µL</td></tr></table>c. Cap the tube, mix, and then briefly centrifuge the contents.</td></tr>
<tr><td>3</td><td>  </td><td>Combine annealed RNA and RT reaction mix</td><td>Add RT reaction mix to the annealed RNA.</td></tr>
<tr><td>4</td><td>  </td><td>Incubate reactions</td><td>a. Incubate the combined reaction mixture at 50°C for 10 minutes.
b. Inactivate the reaction by incubating it at 80°C for 10 minutes.</td></tr>
<tr><td>5</td><td>  </td><td>Remove RNA</td><td>a. Add 1 µL E. coli RNase H and incubate 37°C for 20 minutes.
b. Proceed to PCR amplification (page 5)</td></tr>
</table>

SuperScript™ IV Control Reactions - PCR amplification

Follow the procedure below to perform the PCR amplification step of the SuperScript™ IV RT-PCR control reactions..

The example procedure below shows the appropriate volumes for a single **50-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense the appropriate volumes into each 0.2-mL or 0.5-mL PCR tube before adding template DNA and primers.

<table>
<tr><th colspan="2">Step</th><th>Action</th></tr>
<tr><td>1</td><td>  </td><td>Assemble PCR amplification mix</td><td>a. Prepare two reactions. In each tube, combine the following:
<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>DEPC-treated water</td><td>37.8 µL</td></tr>
<tr><td>10× High Fidelity PCR Buffer</td><td>5 µL</td></tr>
<tr><td>50 mM $MgSO_4$</td><td>2 µL</td></tr>
<tr><td>10 mM dNTP mix (10 mM each)</td><td>1 µL</td></tr>
<tr><td>Control sense primer (10 µM)
(5′-GCTCGTCGTCGACAACGGCTC-3′)</td><td>1 µL</td></tr>
<tr><td>Control antisense primer (10 µM)
(5′-CAAACATGATCTGGGTCATCTTCTC-3′)</td><td>1 µL</td></tr>
<tr><td>cDNA from positive control reaction (step 5, page 4) or
DEPC-treated water for no RT control</td><td>2 µL</td></tr>
<tr><td>Platinum™ Taq DNA Polymerase High Fidelity (5 U/µL)</td><td>0.2 µL</td></tr>
</table>
b. Mix gently by pipetting up and down and briefly centrifuge the components.</td></tr>
<tr><td>2</td><td>  </td><td>Incubate reactions in a thermal cycler</td><td>a. Place reaction mixture in preheated (94°C) thermal cycler.
b. Perform PCR amplification using the following cycling parameters:
<table>
<tr><th colspan="2">Step</th><th>Temperature</th><th>Time</th></tr>
<tr><td colspan="2">Initial denaturation</td><td>94°C</td><td>2 minutes</td></tr>
<tr><td rowspan="3">35 PCR cycles</td><td>Denature</td><td>94°C</td><td>15 seconds</td></tr>
<tr><td>Anneal</td><td>55°C</td><td>30 seconds</td></tr>
<tr><td>Extend</td><td>68°C</td><td>1 minute</td></tr>
<tr><td colspan="2">Hold</td><td>4°C</td><td>hold</td></tr>
</table></td></tr>
<tr><td>3</td><td>  </td><td>Analyze with gel electrophoresis</td><td>Analyze 10 µL of each reaction using agarose gel electrophoresis and ethidium bromide staining.
A 353-bp band should be visible for the positive control reaction with RT. For the no RT control reaction, the same band should be ≤ 50% in intensity when compared to the positive control.</td></tr>
</table>