SuperScript® IV Reverse Transcriptase

Package contents

Catalog Number	Size
18090010	2,000 units
18090050	10,000 units
18090200	4 × 10,000 units

Kit Contents

Storage conditions

Store at –20°C (non-frost-free)

Required materials

- Template: RNA
- Oligo(dT)$_{20}$ primer (Cat. no. 18418-020), random hexamers (Cat. no. N8080127), or 2 µM gene-specific primers
- 10 mM dNTP mix (Cat. no. 18427-013)
- RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. no. 10777-019)
- *E. coli* Ribonuclease H (RNase H) (Cat. no. 18021-014)
- DEPC-treated water (Cat. no. 10813-012)

Timing

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

Selection guides

Go online to view related products.

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 www.lifetechnologies.com/support.

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Print Options

Protocol outline

A. Anneal primer to RNA
B. Assemble reaction mix
C. 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
RNaseOUT™ RNase Inhibitor (40 U/µL)	1.0 µL	µL	2.0 U/µL
50 µM Oligo d(T)$_{20}$ primer, or 50 µM random hexamers, or 2 µM gene-specific primer	1.0 µL 1.0 µL 1.0 µL	µL	2.5 µM 2.5 µM 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 2 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

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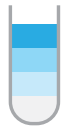
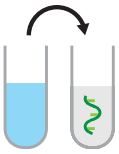
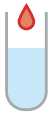
SuperScript® IV First-Strand cDNA Synthesis Reaction

The example procedure below 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.

Steps	Procedure	Procedure details
1 	**Anneal primer to template RNA**	a. Combine the following components in a 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. Component: 50 µM Oligo $d(T)_{20}$ primer, 50 µM random hexamers, or 2 µM gene-specific reverse primer — Volume: 1 µL Component: 10 mM dNTP mix (10 mM each) — Volume: 1 µL Component: Template RNA (10 pg–5 µg total RNA or 10 pg–500 ng mRNA) — Volume: up to 11 µL Component: DEPC-treated or nuclease-free water — Volume: to 13 µL 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.
2 	**Prepare RT reaction mix**	a. Vortex and briefly centrifuge the 5× SSIV Buffer. b. Combine the following components in a reaction tube. Component: 5× SSIV Buffer — Volume: 4 µL Component: 100 mM DTT — Volume: 1 µL Component: RNaseOUT™ Recombinant RNase Inhibitor — Volume: 1 µL Component: SuperScript® IV Reverse Transcriptase (200 U/µL) — Volume: 1 µL c. Cap the tube, mix, and then briefly centrifuge the contents.
3 	**Combine annealed RNA and RT reaction mix**	Add RT reaction mix to the annealed RNA.
4 	**Incubate reactions**	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.
5 	***Optional*: Remove RNA**	**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.
6 	**PCR amplification**	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