

<b>Package contents</b>	<table> <tr> <th>Catalog Number</th><th>Size</th></tr> <tr> <td>18091050</td><td>50 reactions</td></tr> <tr> <td>18091200</td><td>200 reactions</td></tr> </table> Kit Contents	Catalog Number	Size	18091050	50 reactions	18091200	200 reactions
Catalog Number	Size						
18091050	50 reactions						
18091200	200 reactions						
<b>Storage conditions</b>	Store at –20°C (non-frost-free)						
<b>Required materials</b>	<ul style="list-style-type: none"> <li>▪ Template: RNA</li> <li>▪ <i>Optional:</i> 2 µM gene-specific primers</li> </ul>						
<b>Timing</b>	<ul style="list-style-type: none"> <li>▪ Preparation time: 10 minutes</li> <li>▪ Run time: 20 minutes</li> </ul>						
<b>Selection guides</b>	Go online to view related products. <a href="#">PCR Enzymes and Master Mixes</a> <a href="#">RT Enzymes and Kits</a> <a href="#">Real-Time PCR Instruments</a> <a href="#">Real-Time PCR Master Mixes</a> <a href="#">PCR Thermal Cyclers</a>						
<b>Product description</b>	For first strand cDNA synthesis using total RNA or poly(A)+-selected RNA primed with oligo(dT), random primers, or a gene-specific primer.						
<b>Important guidelines</b>	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.						
<b>Online resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.thermofisher.com/support">www.thermofisher.com/support</a> .						

## Protocol outline

- Anneal primer to RNA
- Assemble reaction mix
- 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) <sub>20</sub> 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 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**



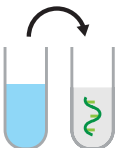

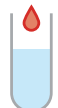

**RT Guidelines**

**Troubleshooting**

**Limited Warranty, Disclaimer,  
and Licensing Information**



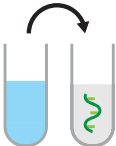

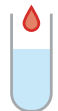
## SuperScript™ IV First-Strand cDNA Synthesis Reaction

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




## 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.

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

## SuperScript™ IV Control Reactions - PCR amplification

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

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