

 Package contents	Catalog Number 18091050, 18091150 18091200, 18091300 Size 50 reactions 200 reactions  Kit Contents
 Storage conditions	Store at –20°C (non-frost-free)
 Required materials	▪ Template: RNA ▪ (<i>Optional</i>) 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

- (*Optional*) Remove genomic DNA
- Anneal primers 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) 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**

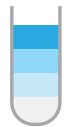
 **Limited Warranty, Disclaimer, and Licensing Information**

©2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Optional: Remove genomic DNA

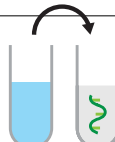
The following example procedure describes the required to remove the genomic DNA (gDNA) from your samples for the RT and No RT Control reactions using the ezDNase™ Enzyme (supplied with Cat. No. 18091150 and 18091300; also available separately at [thermofisher.com](https://www.thermofisher.com)).

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.

Step		Action										
1	 Prepare gDNA digestion reaction mix (on ice)	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>	Component	Volume	10× ezDNase™ Buffer	1 μL	ezDNase™ Enzyme	1 μL	Template RNA (1 pg to 2.5 μg total RNA)	up to 8 μL	Nuclease-free water	to 10 μL
Component	Volume											
10× ezDNase™ Buffer	1 μL											
ezDNase™ Enzyme	1 μL											
Template RNA (1 pg to 2.5 μg total RNA)	up to 8 μL											
Nuclease-free water	to 10 μL											
2	 Digest gDNA	Gently mix and incubate at 37°C for 2 minutes.										
3	 Inactivate ezDNase™ enzyme	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.										
4	 Place the reaction on ice	Chill the tube on ice to bring the sample to room temperature, then briefly centrifuge and place the tube on ice.										

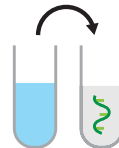
SuperScript™ IV First-Strand cDNA Synthesis Reaction

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.

Step		Action										
1	 Denature template RNA	a. Add 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><thead><tr><th>Component</th><th>Volume</th></tr></thead><tbody><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></tbody></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.	Component	Volume	50 μM Oligo d(T) ₂₀ 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
Component	Volume											
50 μM Oligo d(T) ₂₀ 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											
2	 Prepare RT reaction mix	a. Vortex and briefly centrifuge the 5× SSIV Buffer. b. Add the following components in a reaction tube. <table><thead><tr><th>Component</th><th>Volume</th></tr></thead><tbody><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></tbody></table> c. Cap the tube, mix, and then briefly centrifuge the contents.	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
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											
3	 Combine denatured RNA and RT reaction mix	Add RT reaction mix to the RNA-primer mix from step 1.										
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 <i>E. coli</i> 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										

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.

Step		Action										
1	 Denature template RNA	a. Add the following components in each of two PCR reaction tubes. <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.	Component	Volume	50 μ M Oligo d(T) ₂₀ primer	1 μ L	10 mM dNTP mix (10 mM each)	1 μ L	10 ng/ μ L total HeLa RNA (10 ng total)	1 μ L	DEPC-treated water	10 μ L
Component	Volume											
50 μ M Oligo d(T) ₂₀ primer	1 μ L											
10 mM dNTP mix (10 mM each)	1 μ L											
10 ng/ μ L total HeLa RNA (10 ng total)	1 μ L											
DEPC-treated water	10 μ L											
2	 Prepare RT reaction mix	a. Vortex and briefly centrifuge the 5$\times$ SSIV Buffer. b. Prepare two different control reactions. In each reaction tube, add the following: <table><tr><th>Component</th><th>Volume</th></tr><tr><td>5$\times$ 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.	Component	Volume	5 $\times$ SSIV Buffer	4 μ L	100 mM DTT	1 μ L	Ribonuclease Inhibitor	1 μ L	SuperScript™ IV Reverse Transcriptase (positive control) OR DEPC-treated water (no RT control)	1 μ L
Component	Volume											
5 $\times$ SSIV Buffer	4 μ L											
100 mM DTT	1 μ L											
Ribonuclease Inhibitor	1 μ L											
SuperScript™ IV Reverse Transcriptase (positive control) OR DEPC-treated water (no RT control)	1 μ L											
3	 Combine denatured RNA and RT reaction mix	Add RT reaction mix to the RNA-primer mix from step 1 (one tube for the positive control reaction, one tube for the no RT control reaction).										
4	 Incubate reactions	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.										
5	 Remove RNA	a. Add 1 μL <i>E. coli</i> RNase H and incubate 37°C for 20 minutes. b. Proceed to PCR amplification (page 5)										

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.

Step		Action																						
1	 Assemble PCR amplification mix	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 MgSO₄</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.	Component	Volume	DEPC-treated water	37.8 μL	10× High Fidelity PCR Buffer	5 μL	50 mM MgSO ₄	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 4) or DEPC-treated water for no RT control	2 μL	Platinum™ Taq DNA Polymerase High Fidelity (5 U/μL)	0.2 μL				
Component	Volume																							
DEPC-treated water	37.8 μL																							
10× High Fidelity PCR Buffer	5 μL																							
50 mM MgSO ₄	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 4) or DEPC-treated water for no RT control	2 μL																							
Platinum™ Taq DNA Polymerase High Fidelity (5 U/μL)	0.2 μL																							
2	 Incubate reactions in a thermal cycler	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>	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
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																					
3	 Analyze with gel electrophoresis	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.																						