invitrogen

Package contents

Catalog No.	Size
12594025	25 reaction
12594100	100 reaction
12595025	25 reaction (with ezDNase™ Enzyme)
12595100	100 reaction (with ezDNase™ Enzyme)

Kit contents

Storage conditions

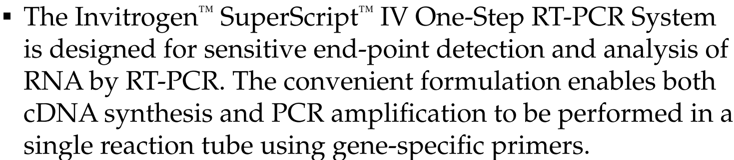
Store all contents at –20°C (non-frost-free)

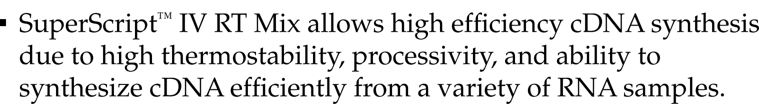
Required materials

- Template: RNA
- Gene specific primers (forward and reverse)

Timing

- Preparation time: 5 minutes
- Total cycling time: varies with target size (1–3.5 hours)





Product description

- The Invitrogen™ SuperScript™ IV One-Step RT-PCR System is designed for sensitive end-point detection and analysis of RNA by RT-PCR. The convenient formulation enables both cDNA synthesis and PCR amplification to be performed in a single reaction tube using gene-specific primers.
- The system can amplify RNA targets up to 14 kb in length, and is compatible with multiplex RT-PCR.
- SuperScript™ IV RT Mix allows high efficiency cDNA synthesis due to high thermostability, processivity, and ability to synthesize cDNA efficiently from a variety of RNA samples.
- 2X Platinum™ SuperFi™ RT-PCR Master Mix contains Platinum™ SuperFi™ DNA Polymerase which has high specificity, offers high yield, and is ideally suited for PCR applications that require sequence accuracy. The master mix uses a buffer system optimized for both RT and PCR reactions in the same tube.
- ezDNase™ enzyme (available separately as **Cat. No. 11766051**) is included with the SuperScript™ IV One-Step RT PCR System with ezDNase™ Kits for protocols that require removal of residual gDNA contamination.

Online resources

Visit our product page for additional information and protocols.

For support, visit **thermofisher.com/support**.

Guidelines for RNA samples

- This kit is optimized for use with 0.01 pg to 1 µg of total RNA, and is compatible with total RNA, mRNA, viral RNA, or *in vitro* transcribed RNA.
- High-quality, intact RNA is essential for RT-PCR, particularly for long targets. RNA must be devoid of RNase contamination and handled using aseptic conditions.
- Isolate total RNA with **TRIzol™ Reagent**, the **PureLink™ RNA Mini Kit**, or the **MagMAX™-96 Total RNA Isolation Kit**.
- Determine RNA quality using a bioanalyzer or by agarose gel electrophoresis.

Guidelines for primers

- Use gene-specific primers (GSPs) with the SuperScript™ IV One-Step RT-PCR System. Oligo(dT) or random primers are not recommended, because nonspecific products can be generated, thereby reducing the amount of target RT-PCR product.
- A final concentration of 0.5 µM for each primer is recommended, but further optimization may be necessary.
- Design primers that anneal to the mRNA sequence in exons on both sides of an intron or exon/exon boundary, to allow differentiation between the amplified cDNA and potential contaminating genomic DNA. If this approach is not feasible, use the protocol that involves residual gDNA removal with ezDNAse™ enzyme.
- Ensure that primers are not self-complementary or complementary to each other at the 3′ ends.
- To calculate primer T_m and estimate appropriate annealing temperatures for PCR, use the T_m calculator at **thermofisher.com/tmcalculator**

Limited product warranty

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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Guidelines for cDNA synthesis and PCR

- Keep all components, reaction mixes, and samples on ice.
- After preparing reaction mixes, transfer them to the preheated thermal cycler and start the RT-PCR program.
- The optimal conditions for RT-PCR depend on primer and target sequences.
- Efficient cDNA synthesis can be accomplished by a 10-minute incubation at 45–60°C. A 50°C incubation is recommended as a general starting point.
- For GC-rich or structurally complex RNA templates, increasing the cDNA synthesis incubation temperatures up to 55–60 °C may improve RT-PCR results.
- Use 40 cycles of amplification for RT-PCR products ≤3 kb, and 35 cycles for RT-PCR products >3 kb.
- For very low RNA input or rare targets, increasing the number of PCR cycles to 40 may improve results.
- The PCR extension time varies with the size of the amplicon (recommended extension time is approximately 30 seconds per 1 kb of amplicon).

SuperScript™ IV One-Step RT-PCR System protocol

Step	Action	Procedure details
1	**Program thermal cycler**	See table below
2	**Prepare RT-PCR reaction mix**	See details below
3	**Run thermal cycler**	Place the reaction in the pre-heated thermal cycler and run program set up in step 1.

Step 1 – Program thermal cycler

Step	Temp	Time	No. cycles
Reverse transcription	45–60°C	10 min	1
RT inactivation/initial denaturation	98°C	2 min	
Amplification	98°C	10 s	35–40 [2]
	55–72°C [1]	10 s	
	72°C	30 s/kb	
Final extension	72°C	5 min	1

[1] **IMPORTANT!** Use the T_m calculator at **thermofisher.com/tmcalculator** to determine actual annealing temperature.
[2] Use 40 cycles for short (≤3 kb) templates only.

Step 2 – Prepare RT-PCR reaction mix

a. Combine the following components in a 0.2-mL, nuclease-free, thin-walled PCR tube on ice.

Component	Volume
2X Platinum™ SuperFi™ RT-PCR Master Mix	25 µL
Forward primer (10 µM)	2.5 µL
Reverse primer (10 µM)	2.5 µL
SuperScript™ IV RT Mix [1]	0.5 µL
Template RNA (0.01 pg to 1 µg total RNA)	varies
Nuclease-free water	to 50 µL

[1] Use all components except the SuperScript™ IV RT Mix for no RT control reactions.

b. Mix gently and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

For support, visit **thermofisher.com/support**.

SuperScript™ IV One-Step RT-PCR System with ezDNase™ Enzyme protocol

Step	Action	Procedure details
1	**Prepare gDNA digestion reaction mix**	Prepare a 10 µL gDNA digestion reaction mix for each RT-PCR reaction. Mix the following components in a 0.2-mL, nuclease-free, thin-walled PCR tube on ice.

Component	Volume
10X ezDNase™ Buffer	1 µL
ezDNase™ Enzyme	1 µL
Template RNA (0.01 pg to 1 µg total RNA)	varies
Nuclease-free Water	to 10 µL

Step	Action	Procedure details
2 (5 min)	**Digest gDNA**	a. Gently mix and incubate at 37°C for 5 minutes. b. (*Optional*) For long RT-PCR products (>3 kb) add 2 µL of 13.2 mM EDTA, then incubate at 65°C for 10 minutes. c. Briefly centrifuge the reaction mix and place the tube on ice.
3	**Program thermal cycler**	

Step	Temp	Time	No. cycles
Reverse transcription	45–60°C	10 min	1
RT inactivation/initial denaturation	98°C	2 min	
Amplification	98°C	10 s	35–40 [2]
	55–72°C [1]	10 s	
	72°C	30 s/kb	
Final extension	72°C	5 min	1

[1] **IMPORTANT!** Use the T_m calculator at **thermofisher.com/tmcalculator** to determine actual annealing temperature.
[2] Use 40 cycles for short (≤3 kb) templates only.

Step	Action	Procedure details
4	**Prepare RT-PCR reaction mix**	a. Combine the following components in the tube on ice containing the template RNA.

Component	Volume
Template RNA (from step 2)	10 µL
2X Platinum™ SuperFi™ RT-PCR Master Mix	25 µL
Forward primer (10 µM)	2.5 µL
Reverse primer (10 µM)	2.5 µL
SuperScript™ IV RT Mix [1]	0.5 µL
Nuclease-free water	to 50 µL

[1] Use all components except the SuperScript™ IV RT Mix for no RT control reactions.

b. Mix gently and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

Step	Action	Procedure details
5	**Run thermal cycler**	Place the reaction in the pre-heated thermal cycler and run program set up in step 3.

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