SuperScript™ One-Step RT-PCR System for Long Templates

Catalog Numbers 11922-010 and 11922-028

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ SuperScript™ One-Step RT-PCR System for Long Templates is designed for the convenient, sensitive, and reproducible detection and analysis of RNA molecules by one-step RT–PCR. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and specific target RNAs from either total RNA or mRNA. Due to the high fidelity of the DNA polymerase blend in the enzyme mix, this kit can also be used for one-step cloning of genes.

The system consists of two major components: SuperScript™ II RT/Platinum™ *Taq* HiFi Mix and 2X Reaction Mix. The enzyme mix is a mixture of SuperScript™ II Reverse Transcriptase, which provides reduced RNase H activity and increased thermal stability, and Platinum™ *Taq* High Fidelity DNA Polymerase , which is a blend of recombinant *Taq* DNA polymerase and the proofreading enzyme *Pyrococcus* species GB–D thermostable polymerase. The *Taq* DNA polymerase in the blend is complexed with a proprietary antibody that inhibits enzyme activity at ambient temperatures. Activity is regained after the denaturation step in PCR cycling at 94°C, providing an automatic "hot start" for *Taq* DNA polymerase in PCR. Hot starts in PCR provide increased sensitivity, specificity, and yield, while allowing assembly of reactions at ambient temperatures.

The 2X Reaction Mix consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, Mg^{2+} optimized for universal use, deoxyribonucleotide triphosphates, and stabilizers. This convenient 2X format allows further addition of template and primer at any desired concentration. Sufficient reagents are provided for 100 amplification reactions of 50 µL each. This enzyme mixture can amplify RNA targets up to 9.0 kb.

Contents and storage

Contents	Cat. No. 11922-010 (25 reactions)	Cat. No. 11922-028 (100 reactions)	Storage
RT/Platinum™ *Taq* HiFi Mix	25 µL	100 µL	Store at –20°C
2X Reaction Mix (a buffer containing 0.4 mM of each dNTP, 2.4 mM $MgSO_4$)	1 mL	3 × 1 mL	
5 mM Magnesium Sulfate	500 µL	500 µL	

Procedural guidelines

Guidelines for RNA

- High-quality intact RNA is essential for successful full-length cDNA synthesis.
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained.
- For total RNA isolation, we recommend the TRIzol™ Reagent (Cat. Nos. 15596-026 and 15596-018). Oligo (dT)–selection for poly(A)+ RNA is typically not necessary, although it may improve the yield of specific cDNAs.

Guidelines for primers

- Gene-specific primers (GSP) are recommended. Use of oligo (dT) or random primers are not recommended as they result in generation of non-specific products in the one-step procedure and the amount of RT–PCR product may be reduced.
- A final primer concentration of 0.2–0.4 μM for each primer is generally optimal. However, for best results, a primer titration using 0.15–0.5 μM is recommended.
- Design primers that anneal to sequence in exons on both sides of an intron or exon/exon boundary of the mRNA to allow differentiation between amplification of cDNA and potential contaminating genomic DNA.
- Primers should not be self-complementary or complementary to each other at the 3′ ends.

Guidelines for magnesium and dNTP concentration

- This system is supplied with a 2X buffer containing 2.4 mM $MgSO_4$ for a final concentration of 1.2 mM, which works well for many targets. The Mg^{2+} concentration can further be optimized (typically up to 2.0 mM final) with the 5 mM $MgSO_4$ solution provided in the kit.
- A 200 μM dNTP concentration is optimal for most RT–PCR reactions.

Guidelines for PCR

- Keep all components, reaction mixes, and samples on ice. After preparation, transfer samples to a pre-heated thermal cycler (45–55°C, depending on the cDNA step temperature) and immediately start the RT–PCR amplification program.
- Efficient cDNA synthesis can be accomplished in a 15–30 minute incubation at 45–55°C.
- SuperScript™ II RT is inactivated, *Taq* DNA polymerase is reactivated, and the RNA/cDNA hybrid is denatured during the 2-minute incubation at 94°C.
- The annealing temperature should be 10°C below the melting temperature of the primers used.
- The extension time varies with the size of the amplicon (approximately 1 minute per 1 kb of amplicon).
- For all targets up to 3 kb, 1 μL of RT/Platinum™ *Taq* HiFi Mix is sufficient.
- As the length of the fragment increases, the need for additional RNA and $MgSO_4$ will also increase.

Guidelines for long RT-PCR

For amplification of long targets up to 12.3 kb, a temperature of 68°C is recommended for the extension steps during PCR. If optimization of the Mg^{2+} concentration is needed, usually 1.2–2.0 mM is sufficient. Increasing the amount of enzyme to 2 μL per reaction, and primers to 0.4 μM can also improve amplification performance of longer RNA targets.

Methods

1. Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.
   Note: The following cycling conditions were established using a DNA Thermal Cycler 9600 or 2400 (Perkin-Elmer) and may have to be altered for other thermal cyclers. Efficient cDNA synthesis can be achieved in a 15–30-minute incubation at 45–55°C. Use a 30-minute incubation at 50°C as a general starting point. Cycling conditions may have to be further optimized for different primers and target sequences.

cDNA synthesis and pre-denaturation		Denature[1]	Anneal	Extend	Final extention (*optional*)
1 CYCLE		35–40 CYCLES			1 CYCLE
45–55°C	94°C	94°C	55–60°C	68°C	72°C
15–30 minutes	2 minutes	15 seconds	30 seconds	1 minute/kb	5–10 minutes

[1] For Perkin Elmer Model 480 cycler, use 30 seconds denaturation instead of 15 seconds.

2. Add the following to the microcentrifuge tubes placed on ice. Reaction cocktails can be made when multiple reactions are being assembled.
   Note: Absence of genomic DNA in RNA preparations can be verified by omitting the RT/Platinum™ *Taq* HiFi Mix and substituting 2 units of *Taq* DNA polymerase in the reaction.

Components	Volume per 50-µL	Final concentration
2X Reaction Mix	25 µL	1X
Template RNA	x µL	10 pg–1 µg
Sense Primer (10 µM)	1 µL	0.2–0.4 µM
Anti-sense Primer (10 µM)	1 µL	0.2–0.4 µM
RT/Platinum™ *Taq* HiFi Mix	1–2 µL	—
Autoclaved distilled water	to 50 µL	—

3. Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed. Depending on the thermal cycler used, overlay with silicone oil, if necessary.
4. Analyze the amplification product.

Troubleshooting

Observation	Possible cause	Recommended action
No amplification product	No cDNA synthesis (temperature too high)	For the cDNA synthesis step, incubate <55°C.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	Not enough starting template RNA	Increase concentration of template RNA; use 100 ng to 1 µg of total RNA.
	RNA has been damaged or degraded	Replace RNA if necessary.
	RT inhibitors are present in RNA	Remove inhibitors in RNA preparation by an additional 70% ethanol wash. Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate, and spermidine.
	Annealing temperature is too high	Decrease temperature as necessary.
	Extension time is too short	Set extension time for at least 60 seconds per kb of target length.
	Cycle number is too low	Increase cycle number.
Low specificity	Reaction conditions not optimal	Optimize magnesium concentration.
		Optimize the primer.
		Optimize the annealing temperature and extension time.
		Increase temperature of RT reaction to 50–55°C.
	Oligo dT or random primers used for 1st strand synthesis	Use gene-specific primers.
Unexpected bands after electrophoresis	RNA contamination with genomic DNA	Pre-treat RNA with DNase I.

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Revision	Date	Description
A.0	28 April 2016	Format, style, and legal updates
—	27 September 2010	Baseline for this revision history

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