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SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase

Catalog. No.	Size	Store at –30°C to –10°C
12574-018	25 reactions	
12574-026	100 reactions	
Publication Part number 12574.pps	MAN0001094	Rev. Date 18 November 2011

Description

The SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase is designed for the sensitive, reproducible, end-point detection and analysis of RNA molecules by RT-PCR. Using this convenient one-step formulation, you can perform both cDNA synthesis and PCR amplification in a single tube using gene-specific primers, and target RNAs from either total RNA or mRNA. The system uses a mixture of SuperScript® III Reverse Transcriptase and Platinum® *Taq* DNA polymerase in an optimized reaction buffer, and it can detect a wide range of RNA targets, from 200 bp to 4.5 kb. The amount of starting material can range from 0.01 pg to 1 µg of total RNA.

SuperScript® III Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability (Kotewicz, 1985; Gerard, 1986). The enzyme can synthesize cDNA at a temperature range of 45–60°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript® III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

Platinum® *Taq* DNA polymerase is recombinant *Taq* DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling at 94°C, providing an automatic "hot start" in PCR for increased sensitivity, specificity, and yield (Chou, 1992; Sharkey, 1994; Westfall, 1997).

The 2X Reaction Mix included in the kit consists of a proprietary buffer system that has been optimized for reverse transcription and PCR, Mg^{2+}, dNTPs, and stabilizers. The convenient 2X format allows you to add template and primer at any desired concentration. A tube of 5 mM $MgSO_4$ is included in the kit for further optimization of the Mg^{2+} concentration. Sufficient reagents are provided for 25 or 100 amplification reactions of 50 µL each.

Note: This kit has been optimized for end-point RT-PCR. For quantitative real-time RT-PCR, use the SuperScript® III Platinum® One-Step Quantitative RT-PCR System (see **Additional Products**, below).

Contents

Store all components at –30°C to –10°C.

Component	25-rxn kit	100-rxn kit
SuperScript® III RT/ Platinum® *Taq* Mix	50 µL	200 µL
2X Reaction Mix (a buffer containing 0.4 mM of each dNTP, 3.2 mM $MgSO_4$)	1 mL	3 × 1 mL
5-mM Magnesium Sulfate	500 µL	500 µL

Additional Products

The following products are also available from Life Technologies.

Product	Amount	Catalog no.
SuperScript® III Platinum® One-Step Quantitative RT-PCR System	100 rxns	11732-020
	500 rxns	11732-088
TRIzol® Reagent	100 mL	15596-026
	200 mL	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	to order, visit www.lifetechnologies.com	

Product Use: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Important Parameters and Guidelines

RNA

- High quality intact RNA is essential for successful full-length cDNA synthesis.
- For low copy-number genes or longer targets, use more starting material (>10 ng total RNA).
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained.
- We recommend TRIzol® Reagent for isolation of total RNA (Chomcyznski, 1987; Chirgwin, 1979). See page 1 for ordering information. Oligo(dT) selection for poly(A)$^+$ RNA is typically not necessary, although it may improve the yield of specific cDNAs.

Primers

- We recommend using gene specific primers (GSPs). We do not recommend using oligo (dT) or random primers, because they can generate nonspecific products in the one-step procedure and the amount of RT-PCR product may be reduced.
- A final primer concentration of 0.2 µM for each primer is generally optimal. However, for best results, we recommend performing a primer titration of 0.15–0.5 µM.
- 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.
- Primers should not be self-complementary or complementary to each other at the 3′ ends.

Magnesium and dNTP concentration

- $MgSO_4$ is included in the 2X Reaction Mix at a final concentration of 1.6 mM, which works well for most targets (Sitaraman, 1997). If needed, the magnesium concentration can further be optimized (usually between 1.4–2 mM) with the 5-mM $MgSO_4$ provided in the kit.
- dNTPs are included in the 2X Reaction Mix at a final concentration of 200 µM, which is optimal for most reactions.

Reactions

- Program the thermal cycler before setting up the reaction. The thermal cycler should be preheated to 45–60°C, depending on the temperature selected for cDNA synthesis.
- For difficult or high GC-content templates, use a 60°C cDNA synthesis temperature.
- Keep all components, reaction mixes, and samples on ice. After preparation of the samples, transfer them to the preheated thermal cycler and immediately start the RT-PCR program.
- Efficient cDNA synthesis can be accomplished in a 15–30 minutes incubation at 45–60°C. For small targets, an incubation time of 5 minutes may be sufficient.
- SuperScript® II RT is inactivated, Platinum® *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 4.5 kb, 2 µL of SuperScript® III RT/ Platinum® *Taq* Mix is sufficient.

Protocol

The following cycling conditions were established and tested using a GeneAmp® PCR System 9600 and 2400 and a DNA Engine® PTC-200. You may need to adjust these conditions for other thermal cyclers.

Efficient cDNA synthesis can be achieved in a 15–30 minute incubation at 45–60°C. We recommend a 30-minute incubation at 55°C as a general starting point. The optimal temperature for reverse transcription will depend on primer and target sequences. Cycling conditions may have to be further optimized for different sequences. Three-step cycling (separate annealing and extension steps) is required.

1. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification, as follows:

   cDNA synthesis

   1 cycle: 45–60°C for 15–30 minutes

   Denaturation

   1 cycle: 94°C for 2 minutes

   PCR amplification

   40 cycles: 94°C for 15 seconds (denature)
   55-65°C for 30 seconds (anneal)
   68°C for 1 minute per kilobase (extend)

   Final extension (optional)

   1 cycle: 68°C for 5 minutes

2. Add the following to a 0.2-mL, nuclease-free, thin-walled PCR tube on ice. For multiple reactions, you can prepare a master mix to minimize reagent loss and enable accurate pipetting.

Component	Volume
2X Reaction Mix	25 µL
Template RNA (0.01 pg–1 µg)	x µL
Sense primer (10 µM)	1 µL
Anti-sense primer (10 µM)	1 µL
SuperScript® III RT/ Platinum® *Taq* Mix*	2 µL
Autoclaved distilled water	to 50 µL

*You can verify absence of genomic DNA in RNA preparations by omitting the SuperScript® III RT/Platinum® *Taq* Mix and substituting 2 units of Platinum® *Taq* DNA polymerase in the reaction.

3. Gently mix, making 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. Place the reaction in the preheated thermal cycler programmed as described above. Collect the data and analyze the results.

Troubleshooting Guide

Problem	Possible cause	Possible solution
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 the 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 the RNA preparation by an additional 70% ethanol wash. **Note**: Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine (Berger, 1987, Gerard, 1994).
	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 60°C.
	Oligo(dT) or random primers used for first-strand synthesis	Use only gene-specific primers.
Unexpected bands after electrophoretic analysis	Contamination by genomic DNA	Pretreat RNA with DNase I, Amplification Grade (Cat. no. 18068-015), as described in the DNase I documentation. Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA. To test if products were derived from DNA, substitute 2 units of Platinum® Taq DNA polymerase for the SuperScript® III RT/Platinum® Taq mix in the reaction
	Nonspecific annealing of primers	Vary the annealing temperature, Optimize the magnesium concentration for each template and primer combination.
	Primers formed dimers	Design primers without complementary sequences at the 3´ ends.

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Product Qualification and SDS

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