SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit with ROX

Cat. no. 11746-100 **Size: 100 reactions**
Cat. no. 11746-500 **Size: 500 reactions**

Store at –20°C

Description

The SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit with ROX a one-step quantitative RT-PCR system for use with instruments that support normalization with ROX Reference Dye. This system combines SuperScript™ III Reverse Transcriptase (RT) and Platinum® *Taq* DNA Polymerase in a single enzyme mix, with SYBR® Green I fluorescent dye and ROX Reference Dye in a separate 2X reaction mix. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. Reagents are provided for 100 or 500 amplification reactions of 50 µl each.

The system enables highly sensitive detection from as few as 10 copies of a target gene, with a broad dynamic range that supports accurate quantification of high-copy mRNA from up 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 (1). The enzyme can synthesize cDNA at a temperature range of 42–60°C. 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 (2, 3). Activity is restored after the denaturation step in PCR cycling, providing an automatic "hot start" in PCR for increased sensitivity, specificity, and yield.
- **2X SYBR® Green Reaction Mix** consists of a proprietary buffer system, SYBR® Green I, ROX Reference Dye, $MgSO_4$, dNTPs, and stabilizers. SYBR® Green I is a fluorescent dye that binds directly to double-stranded DNA (dsDNA). In qPCR, as dsDNA accumulates, the dye generates a signal that is proportional to the DNA concentration and that can be detected using real-time qPCR instruments (5, 6). SYBR® Green I in this formulation can quantify as few as 10 copies of a target gene in as little as 0.1 pg of total RNA, has a broad dynamic range of six orders of magnitude, and is compatible with melting curve analysis.
- **ROX Reference Dye** is included at a final concentration of 500 nM to normalize the fluorescent signal on instruments that are compatible with this option. ROX can adjust for non-PCR-related fluctuations in fluorescence between reactions, and provides a stable baseline in multiplex reactions. It is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester.

RNaseOUT™ Ribonuclease Inhibitor is included in the SuperScript™ III RT/Platinum® Taq Mix to safeguard against degradation of target RNA due to ribonuclease contamination. A tube of 50-mM $MgSO_4$ is included in the kit for further optimization of the Mg^{2+} concentration.

Note: For instruments that are not compatible with ROX or require ROX at a lower concentration, we recommend the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit (see **Additional Products** below).

Component	100-Rxn Kit	500-Rxn Kit
SuperScript™ III RT/Platinum® Taq Mix (includes RNaseOUT™)	100 µl	500 µl
2X SYBR® Green Reaction Mix with ROX (includes 0.4 mM of each dNTP, 6 mM $MgSO_4$, and 1 µM ROX)	2 × 1.25 ml	12.5 ml
50-mM Magnesium Sulfate ($MgSO_4$)	1 ml	2 × 1 ml

Storage

Store components in the dark at –20ºC.

Additional Products

Product	Amount	Catalog no.
SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit	100 rxns	11736-051
	500 rxns	11736-059
PureLink™ Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
DNase I, Amplification Grade	100 units	18068-015
Custom Primers	visit www.invitrogen.com/oligos	

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Recommendations and Guidelines for One-Step qRT-PCR

Instrument Compatibility

This kit can be used with real-time instruments that are compatible with ROX Reference Dye at a final concentration of 500 nM. These instruments include the ABI PRISM® 7000, 7700, and 7900HT; the ABI 7300 Real-Time PCR System; and the ABI GeneAmp® 5700.

Note: This kit is *not* compatible with instruments that use ROX at a final concentration lower than 500 nM, including the ABI 7500 and the Stratagene Mx3000P®, Mx3005P™, and Mx4000®. For these instruments, we recommend the SuperScript™ III Platinum® One-Step Quantitative RT-PCR System, which includes ROX as a separate tube that can be added at the required concentration (see **Additional Products**, page 1).

Template

Starting material can range from 1 pg to 1 µg of purified total RNA. If you are using purified mRNA, the amount of template may be as low as 0.5 pg. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with DNase I (Catalog no. 18068-015) to remove any contaminating genomic DNA.

To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System (catalog no. 12183-018), TRIzol® Reagent (catalog nos. 15596-026 and 15596-018), or the PureLink™ 96 Total RNA Purification Kit for high-throughput applications (catalog no. 12173-011).

Magnesium Concentration

The 2X SYBR® Green Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the separate tube of 50-mM magnesium sulfate to increase the magnesium concentration. Use the following table to determine the amount of $MgSO_4$ to add to achieve the specified concentration (in a 50-µl PCR with 25 µl of 2X SYBR® Green Reaction Mix):

For a Final $MgSO_4$ Concentration of	Add this Volume of 50-mM $MgSO_4$ (per 50-µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl

Decrease the amount of water in the reaction accordingly.

Melting Curve Analysis

Melting curve analysis should always be performed during qRT-PCR to identify the presence of primer dimers and analyze the specificity of the reaction. For more information, visit www.invitrogen.com/qpcr.

Primers

Gene-specific primers are required. We strongly recommend using a primer design program such as OligoPerfect™, available on the Web at www.invitrogen.com/oligos, or Vector NTI™. The amplicon length should be approximately 80–250 bp, and the primers should be designed to anneal to exons on both sides of an intron or within the exon/exon boundary of the mRNA to allow differentiation of cDNA from genomic DNA.

A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 500 nM.

Reaction Setup and Conditions

Keep all components, reaction mixes and samples on ice. For most templates, efficient cDNA synthesis can be accomplished in a 3-minute incubation at 50°C. For problematic templates, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature up to 60°C. The cDNA synthesis temperature can range from 42°C to 60°C.

Quality Control

The product is tested functionally by qRT-PCR using total HeLa RNA as template. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration and detection of β-actin mRNA in 1 pg of total HeLa RNA.

General Protocol for ABI Instruments

Follow the protocol below for one-step qRT-PCR on compatible ABI real-time instruments (see **Instrument Compatibility**, page 2). A standard 50-µl reaction size is provided; component volumes can be scaled as desired (*e.g.*, scaled down to a 20-µl reaction volume for 384-well plates).

1. Program your real-time instrument to perform cDNA synthesis immediately followed by PCR amplification, as shown below. Optimal temperatures and incubation times may vary for different target sequences.

   cDNA synthesis: 50°C for 3 minutes hold (cDNA synthesis temperature may range from 42–60°C)
   PCR: 95°C for 5 minutes hold
   40 cycles of:
   95°C for 15 seconds
   60°C for 30 seconds
   40°C, 1 minute
   Melting curve analysis: Refer to instrument documentation

2. Set up reactions **on ice**. Volumes for a single 50-µl reaction are listed below. For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (*e.g.*, template). **Note:** Preparation of a master mix is *crucial* in qRT-PCR to reduce pipetting errors.

Component	Single rxn
SuperScript™ III RT/Platinum® Taq Mix (includes RNaseOUT™)	1 µl*
2X SYBR® Green Reaction Mix with ROX	25 µl
Forward primer, 10 µM	1 µl
Reverse primer, 10 µM	1 µl
Template (1 pg to 1 µg total RNA)	≤ 10 µl
DEPC-treated water	to 50 µl

   *To test for genomic DNA contamination of the RNA template, prepare a control reaction containing 2 units of Platinum® *Taq* DNA Polymerase (catalog no. 10966-018) instead of the SuperScript™ III RT/Platinum® Taq Mix.

   **See the table on page 2 for the amount/concentration of ROX to use for your specific instrument.

3. Cap or seal the reaction tube/PCR plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.

4. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.

References

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Troubleshooting Guide

Problem	Possible Cause	Solution
No amplification product; Relative fluorescent signal ≤ background or no-template control	cDNA synthesis temperature too high, low priming efficiency	Lower incubation temperature.
	RT or cDNA primer blocked by secondary structure	Raise incubation temperature. Redesign primer(s).
	RNA has been damaged/degraded	Replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
Poor sensitivity	Not enough template RNA	Increase concentration of template RNA; use 10 ng–1 µg total RNA.
Product detected at higher than expected cycle number	RNA has been damaged/degraded	Replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	RT inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine.
	Inefficient cDNA synthesis	Adjust cDNA synthesis temperature and/or primer design. Double the amount of reverse primer (*e.g.*, to 400 nM).
	Inefficient PCR amplification	Optimize PCR conditions: Adjust annealing temperature as necessary. Increase magnesium concentration. Redesign primers.
Higher than expected signal	Too much sample added to reactions	Decrease the concentration of template RNA.
Product detected at lower-than-expected cycle number, and/or positive signal from no-template controls	Template or PCR carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-PCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipet tips or positive displacement pipettors.
Unexpected bands after electrophoresis	Genomic DNA contamination	Pre-treat RNA with DNase I.
	Oligo(dT) or random primers used for cDNA synthesis	Use only gene-specific primers.
	Low specificity in PCR	Optimize PCR conditions as described above.

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