

## SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR Kit

Cat. no. 11736-051  
Cat. no. 11736-059

Size: 100 reactions  
Size: 500 reactions  
Store at -20°C

### Description

The SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR kit is a one-step quantitative RT-PCR kit for the detection and quantification of RNA using real-time detection instruments. This system combines SuperScript® III Reverse Transcriptase (RT) and Platinum® *Taq* DNA Polymerase in a single enzyme mix, with SYBR® Green I fluorescent 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, MgSO<sub>4</sub>, 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.

RNaseOUT™ Ribonuclease Inhibitor is included in the enzyme mix to safeguard against degradation of target RNA due to ribonuclease contamination. A tube of 50-mM MgSO<sub>4</sub> is included in the kit for further optimization of the Mg<sup>2+</sup> concentration. ROX Reference Dye is included as a separate component to normalize the fluorescent signal between reactions, for instruments that are compatible with this option. BSA is provided as a separate component for optimization on the Roche LightCycler®.

<b>Component</b>	<b>100-Rxn Kit</b>	<b>500-Rxn Kit</b>
SuperScript® III RT/Platinum® <i>Taq</i> Mix (includes RNaseOUT™ Ribonuclease Inhibitor)	100 µl	500 µl
2X SYBR® Green Reaction Mix (includes 0.4 mM of each dNTP and 6 mM MgSO <sub>4</sub> )	2 × 1.25 ml	12.5 ml
50-mM Magnesium Sulfate (MgSO <sub>4</sub> )	1 ml	2 × 1 ml
ROX Reference Dye (25 µM)	100 µl	500 µl
20X Bovine Serum Albumin (ultrapure, non-acetylated) (1 mg/ml)	300 µl	1.3 ml

### Storage

Store components at -20°C. Stability can be extended by storing at -80°C. ROX Reference Dye must be stored in the dark.

### Additional Products

The following products are also available from Invitrogen.

<b>Product</b>	<b>Amount</b>	<b>Catalog no.</b>
SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR Kit with ROX	100 rxns	11746-100
	500 rxns	11746-500
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
Fluorescein NIST-Traceable Standard (50 µM)	5 × 1 ml	F36915
Custom Primers	visit <a href="http://www.invitrogen.com/oligos">www.invitrogen.com/oligos</a>	

## Recommendations and Guidelines for One-Step qRT-PCR

### Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the ABI PRISM® 7000, 7700, and 7900HT; the ABI 7300 and 7500 Real-Time PCR Systems; the ABI GeneAmp® 5700; the Bio-Rad iCycler™; the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Corbett Research Rotor-Gene™; the MJ Research DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector; and the Cepheid Smart Cycler®. For instrument-specific protocols, go to [www.invitrogen.com/qpcr](http://www.invitrogen.com/qpcr). Optimal cycling conditions will vary with different instruments.

### 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<sub>4</sub> 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 <sub>4</sub> Concentration of	Add this Volume of 50-mM MgSO <sub>4</sub> (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.

### ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 25 µM concentration, and is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester in 20 mM Tris-HCl (pH 8.4), 0.1 mM EDTA, and 0.01% Tween® 20. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 50-µl reaction	Final ROX Conc.
ABI 7000, 7300, 7700, 7900HT	1.0 µl	500 nM
ABI 7500; Stratagene Mx3000™, Mx3005P™, Mx4000™	0.1 µl*	50 nM

\*To accurately pipet 0.1 µl per reaction, dilute ROX 1:10 immediately before use and use 1 µl of the dilution.

Note that the SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR Kit with ROX includes ROX in the 2X SYBR® Green Reaction Mix at a final concentration of 500 nM (see **Additional Products**, page 1).

### Fluorescein

The Bio-Rad iCycler™ requires the use of fluorescein as a reference dye to normalize the fluorescent reporter signal in SYBR® Green reactions. Fluorescein NIST-Traceable Standard is available from Invitrogen as a 50-µM solution (see **Additional Products**, page 1). We recommend using a final concentration of 50 nM as a general starting point. Optimal results may require a titration between 10 and 100 nM.

### 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](http://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](http://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.

### Bovine Serum Albumin (BSA)

BSA (ultrapure, non-acetylated) is included as a separate tube in each kit for use in LightCycler® reactions. For more information, visit [www.invitrogen.com/qpcr](http://www.invitrogen.com/qpcr).

### 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.

## General Protocol for ABI Instruments

Follow the protocol below for one-step qRT-PCR on ABI real-time instruments. Note the separate cycling conditions for the ABI 7500 in Fast Mode, and the lower amount of ROX Reference Dye required for the ABI 7500 systems. This generic protocol may also be used for other real-time instruments.

For more instrument-specific protocols, go to [www.invitrogen.com/qpcr](http://www.invitrogen.com/qpcr). A standard 50- $\mu$ l reaction size is provided; component volumes can be scaled as desired (*e.g.*, scaled down to a 20- $\mu$ l 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.

Standard Cycling Program	Fast Cycling Program (for the ABI 7500 in Fast Mode)
50°C for 3 minutes hold (cDNA synthesis temperature may range from 42–60°C)	Select <i>Fast Mode</i> on the Thermal Profile tab
95°C for 5 minutes hold	50°C for 5 minutes hold (cDNA synthesis temperature may range from 42–60°C)
40 cycles of:	95°C for 2 minutes hold
95°C for 15 seconds	40 cycles of:
60°C for 30 seconds	95°C, 3 seconds
40°C, 1 minute	60°C, 30 seconds
Melting curve analysis: Refer to instrument documentation	Melting curve analysis: Refer to instrument documentation

2. Set up reactions **on ice**. Volumes for a single 50- $\mu$ l 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 $\mu$ l*
2X SYBR® Green Reaction Mix	25 $\mu$ l
Forward primer, 10 $\mu$ M	1 $\mu$ l
Reverse primer, 10 $\mu$ M	1 $\mu$ l
ROX Reference Dye (optional)	1 $\mu$ l/0.1 $\mu$ l**
Template (1 pg to 1 $\mu$ g total RNA)	≤ 10 $\mu$ l
DEPC-treated water	to 50 $\mu$ l

\*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.

## Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at [www.invitrogen.com/cofa](http://www.invitrogen.com/cofa), and is searchable by product lot number, which is printed on each box.

## References

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## Troubleshooting Guide

Problem	Possible Cause	Solution
No amplification product; Relative fluorescent signal $\leq$ background or no-template control	cDNA synthesis temperature too high, low priming efficiency RT or cDNA primer blocked by secondary structure RNA has been damaged/degraded RNase contamination	Lower incubation temperature.  Raise incubation temperature. Redesign primer(s).  Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor.
Poor sensitivity	Not enough template RNA	Increase concentration of template RNA; use 10 ng–1 $\mu$ g total RNA.
Product detected at higher than expected cycle number	RNA has been damaged/degraded RNase contamination RT inhibitors are present in RNA  Inefficient cDNA synthesis  Inefficient PCR amplification	Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor. 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. Adjust cDNA synthesis temperature and/or primer design. Double the amount of reverse primer (e.g., to 400 nM).  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 Oligo(dT) or random primers used for cDNA synthesis  Low specificity in PCR	Pre-treat RNA with DNase I. Use only gene-specific primers.  Optimize PCR conditions as described above.

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