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# SuperScript® III Platinum® One-Step Quantitative RT-PCR System with ROX

**Cat. no. 11745-100** **Size: 100 reactions**
**Cat. no. 11745-500** **Size: 500 reactions**

**Store at -20°C**

## Description

SuperScript® III Platinum® One-Step Quantitative RT-PCR System with ROX is a one-step, quantitative RT-PCR (qRT-PCR) kit for use with real-time 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. 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 RNA template, with a broad dynamic range that supports accurate quantification of high-copy mRNA at 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 Reaction Mix with ROX** consists of a proprietary buffer system, $MgSO_4$, dNTPs, ROX Reference Dye, and stabilizers. ROX 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.

This one-step qRT-PCR kit has been formulated for use with fluorogenic primers (*e.g.*, LUX™ Primers) or fluorogenic probe-based technology (*e.g.*, TaqMan® probes) (4–7). For one-step qRT-PCR using SYBR® Green I dye and premixed ROX, we recommend the SuperScript® III Platinum® SYBR® Green One-Step qPCR System with ROX (Catalog nos. 11746-100 and 11746-500).

For instruments that are not compatible with ROX or require ROX at a lower concentration, we recommend the SuperScript® III Platinum® One-Step Quantitative RT-PCR System (see **Additional Products** below).

| Component | 100-Rxn Kit | 500-Rxn Kit |
|---|---|---|
| SuperScript® III RT/Platinum® *Taq* Mix | 100 µl | 500 µl |
| 2X Reaction Mix with ROX (contains 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 -20°C.

## Additional Products

The following products are also available from Invitrogen.

| Product | Amount | Catalog no. |
|---|---|---|
| SuperScript® III Platinum® One-Step Quantitative RT-PCR System | 100 rxns | 11732-020 |
| | 500 rxns | 11732-088 |
| PureLink™ RNA Mini Kit | 50 rxns | 12183018A |
| TRIzol® Reagent | 100 ml | 15596-026 |
| | 200 ml | 15596-018 |
| RNaseOUT™ Recombinant Ribonuclease Inhibitor | 5000 units | 10777-019 |
| DNase I, Amplification Grade | 100 units | 18068-015 |
| LUX™ Fluorogenic Primers | visit www.invitrogen.com/dluxdesigner | |

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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, 7900HT, and 7900HT Fast; 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 7500 Fast, 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 reduced to 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.

**Optional:** An RNase inhibitor such as RNaseOUT™ (Cat. No. 10777-019) may be added to the reaction after the 2X Reaction Mix to safeguard against degradation of target RNA due to ribonuclease contamination.

### Isolating Total RNA

To isolate total RNA, we recommend the PureLink™ RNA Mini Kit (Cat. no. 12183018A), TRIzol® Reagent (Cat. nos. 15596-026 and 15596-018), or the PureLink™ 96 Total RNA Purification Kit for high-throughput applications (Cat. no. 12173-011). Isolation of mRNA from total RNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.

### Magnesium Concentration

The 2X 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 Reaction Mix):

| Volume of 50-mM $MgSO_4$ (per 50-µl Rxn) | Final $MgSO_4$ Conc. |
|---|---|
| 1 µl | 4.0 mM |
| 2 µl | 5.0 mM |
| 3 µl | 6.0 mM |

Decrease the amount of water in the reaction accordingly.

### Primers

Gene-specific primers are required. LUX™ Fluorogenic Primers (www.invitrogen.com/lux) are available separately from Invitrogen, and may be designed for specific targets using the D-LUX™ Designer at www.invitrogen.com/dluxdesigner.

A final concentration of 200 nM per primer is effective for most reactions. Doubling the amount of reverse primer (to 400 nM) may improve the performance of certain reactions. Optimal results may require a primer titration between 100 and 500 nM.

### Dual-Labeled Probes

A final probe concentration of 100 nM is effective for most reactions. The optimal concentration may vary between 50 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 15-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.

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## General Protocol for ABI Instruments

Follow the protocol below for one-step qRT-PCR using either LUX™ Primers or TaqMan® Probes 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:

   50°C for 15 minutes hold (cDNA synthesis temperature may range from 42–60°C)
   95°C for 2 minutes hold
   40 cycles of:
   - 95°C, 15 seconds
   - 60°C, 30 seconds (60 seconds for the 7900HT)

   Melting curve analysis (LUX™ Primers only): 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.

| **LUX™ Primers Reaction Mix** | | **TaqMan® Probes Reaction Mix** | |
|---|---|---|---|
| Component | Single rxn | Component | Single rxn |
| SuperScript® III RT/Platinum® *Taq* Mix | 1 µl* | SuperScript® III RT/Platinum® *Taq* Mix | 1 µl* |
| 2X Reaction Mix with ROX | 25 µl | 2X Reaction Mix with ROX | 25 µl |
| LUX™ labeled primer, 10 µM | 1 µl | Forward primer, 10 µM | 1 µl |
| Unlabeled primer, 10 µM | 1 µl | Reverse primer, 10 µM | 1 µl |
| RNaseOUT™ (optional) | 1 µl | Fluorogenic probe, 10 µM | 0.5 µl |
| Template (1 pg to 1 µg total RNA) | ≤ 10 µl | RNaseOUT™ (optional) | 1 µl |
| DEPC-treated water | to 50 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.

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 provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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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. |
| | Fluorescent probe not functional | Validate probe design and presence of fluorophore and quencher: Treat TaqMan® probe with DNase, and check for increase in fluorescence. Redesign and/or resynthesize probe if necessary. |
| 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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