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

**Cat. no. 11732-020** **Size: 100 reactions**
**Cat. no. 11732-088** **Size: 500 reactions**

**Store at –20°C**

## Description

The SuperScript® III Platinum® One-Step Quantitative RT-PCR System is a one-step, quantitative RT-PCR (qRT-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. 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°C –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** consists of a proprietary buffer system, $MgSO_4$, dNTPs, and stabilizers. A tube of 50 mM $MgSO_4$ is included in the kit for further optimization of the $Mg^{2+}$ concentration.

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, we recommend the SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR System (see **Additional products**, below).

**Note:** This kit has been optimized for real-time qRT-PCR. For end-point RT-PCR, we recommend the SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase (Catalog nos. 12574-018 and 12574-026).

| Component | 100 reaction kit | 500 reaction kit |
|---|---|---|
| SuperScript® III RT/Platinum® *Taq* Mix | 100 µL | 500 µL |
| 2X Reaction Mix (a buffer containing 0.4 mM of each dNTP and 6 mM $MgSO_4$) | 2 × 1.25 mL | 12.5 mL |
| 50 mM Magnesium Sulfate ($MgSO_4$) | 1 mL | 2 × 1 mL |
| ROX™ Reference Dye (25 µM) | 100 µL | 500 µL |

## Storage

Store components at –20ºC. Stability can be extended by storing at –80ºC. ROX™ Reference Dye must be stored in the dark.

## Additional products

The following products are also available. Visit **www.lifetechnologies.com**

| Product | Amount | Catalog no. |
|---|---|---|
| SuperScript® III Platinum® One-Step Quantitative RT-PCR System with ROX™ Reference Dye | 100 reactions | 11745-100 |
| | 500 reactions | 11745-500 |
| SuperScript® III Platinum® SYBR® Green One-Step qPCR System | 100 reactions | 11736-051 |
| | 500 reactions | 11736-059 |
| PureLink® Micro-to-Midi™ Total RNA Purification System | 50 reactions | 12183-018 |
| 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.lifetechnologies.com** |

## 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 Applied Biosystems 7000, 7300, 7500, 7700, and 7900HT Real-Time PCR Systems; the Applied Biosystems 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 Cepheid Smart Cycler® thermal cyclers. For instrument-specific protocols, go to **www.lifetechnologies.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 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.

### Isolate total RNA

To isolate total RNA, we recommend the PureLink™ Micro-to-Midi™ Total RNA Purification System (Cat. no. 12183-018), 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 mM 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.

### 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™ Reference Dye is supplied at a 25 µM concentration, and is composed of a glycine conjugate of 5-carboxy-X-rhodamine, succinimidyl ester in 10 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™ Reference Dye to use with a particular instrument:

| Instrument | ROX™ dye per 50-µL reaction | Final ROX™ dye concentration |
|---|---|---|
| Applied Biosystems 7000, 7300, 7700, 7900HT, and 7900HT Fast | 1.0 µL | 500 nM |
| Applied Biosystems 7500; Stratagene Mx3000™, Mx3005P™, and Mx4000 | 0.1 µL* | 50 nM |

*To accurately pipet 0.1 µL per reaction, we recommend that you dilute ROX™ Reference Dye 1:10 immediately before use and use 1 µL of the dilution.

Note that the **SuperScript® III Platinum® One-Step Quantitative RT-PCR System with ROX™ Reference Dye** includes ROX™ Reference Dye in the 2X Reaction Mix at a final concentration of 500 nM (see **Additional products**, page 1).

### Primers

Gene-specific primers are required. LUX™ Fluorogenic Primers (**www.lifetechnologies.com**) are available separately.

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 mM 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 mM 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.

## General protocol for ABI instruments

Follow the protocol below for one-step qRT-PCR using either LUX™ primers or TaqMan® probes 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 and 7500 Fast systems. This generic protocol may also be used for other real-time instruments.

For more instrument-specific protocols, go to **www.lifetechnologies.com/qpcr**. 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.

| **Standard cycling program** | **Fast cycling program (for the ABI 7500 in Fast Mode)** |
|---|---|
| 50°C for 15 minutes hold (cDNA synthesis temperature may range from 42°C–60°C) | Select *Fast Mode* on the Thermal Profile tab |
| 95°C for 2 minutes hold | 50°C for 5 minutes hold |
| 40 cycles of: | 95°C for 2 minutes hold |
| 95°C, 15 seconds | 40 cycles of: |
| 60°C, 30 seconds (60 seconds for the 7900HT) | 95°C, 3 seconds |
| | 60°C, 30 seconds |
| Melting curve analysis (LUX™ primers only): Refer to instrument documentation. | 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 | 25 µL | 2X Reaction Mix | 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 |
| ROX™ Reference Dye (optional) | 1 µL/0.1 µL** | Fluorogenic probe, 10 µM | 0.5 µL |
| RNaseOUT™ (optional) | 1 µL | ROX™ Reference Dye (optional) | 1 µL/0.1 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.

**See the table on page 2 for the amount/concentration of ROX™ Reference Dye 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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3. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., and Daiss, J.L. (1994) Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. *BioTechnology 12*, 506.
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5. Tyagi, S. and Kramer, F.R. (1996) Molecular beacons: probes that fluoresce upon hybridization. *Nature Biotechnology 14*, 303.
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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:<br>• Adjust annealing temperature as necessary.<br>• Increase magnesium concentration.<br>• 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).<br>• Use separate dedicated pipettors for reaction assembly and post-PCR analysis.<br>• Assemble reactions (except for target addition) in a DNA-free area.<br>• 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. |

### Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available from **www.lifetechnologies.com**.

### Important Licensing Information

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