SuperScript™ III Platinum™ One-Step qRT-PCR Kit

Catalog Numbers 11732-020 and 11732-088

Doc. Part No. 11732.pps **Pub. No.** MAN0001006 **Rev.** C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ SuperScript™ III Platinum™ One-Step qRT-PCR Kit 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. 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. 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 or fluorogenic probe-based technology (e.g., TaqMan™ probes). For one-step qRT–PCR using SYBR™ Green I dye, we recommend the SuperScript™ III Platinum™ SYBR™ Green One–Step qRT–PCR System (see "Ordering information" on page 4).

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 (Cat. Nos. 12574-018 and 12574-026).

Contents and storage

Contents	Cat. No. 11732-020 (100 reactions)	Cat. No. 11732-088 (500 reactions)	Storage
SuperScript™ III RT/Platinum™ *Taq* Mix	100 µL	500 µL	Store components at –20°C. Stability can be extended by storing at –80°C. ROX™ Reference Dye must be stored in the dark.
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	

Procedural guidelines

Guidelines for 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 Agilent™ Mx3000P™, Mx3005P™, and Mx4000™; the Corbett Research Rotor-Gene™; the MJ Research DNA Engine Opticon™, Opticon™ 2, and Chromo4™ Real-Time Detector; and Cepheid SmartCycler™ thermal cyclers. For instrument-specific protocols, go to the Real-Time PCR page on our website (**thermofisher.com**). Optimal cycling conditions will vary with different instruments.

Guidelines for the 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 (Cat. 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.

Guidelines for total RNA isolation

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.

Guidelines for 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 3–6 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) and decrease the amount of water in the reaction accordingly.

Volume of 50 mM $MgSO_4$ (per 50- µL reaction)	Final $MgSO_4$ concentration
1 µL	4.0 mM
2 µL	5.0 mM
3 µL	6.0 mM

Guidelines for 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, 7900HT Fast, StepOne™, and StepOnePlus™ systems	1.0 µL	500 nM
Applied Biosystems™ 7500, ViiA™ 7, and QuantStudio™ 3, 5, 6 Flex, 7 Flex, and 12k Flex systems; Agilent™ Mx3000P™, Mx3005P™, and Mx4000™	0.1 µL[1]	50 nM

[1] 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: 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 "Ordering information" on page 4).

Guidelines for primers

- Gene-specific primers are required, such as those found in Applied Biosystems™ TaqMan™ Gene Expression Assays (visit **thermofisher.com**).
- 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.

Guidelines for 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.

Guidelines for reaction setup

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 42–60°C.

Perform one-step qRT-PCR

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

For more instrument-specific protocols, see **thermofisher.com/support**. 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 7500 system in Fast Mode)
• 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 system)	• Select Fast Mode on the Thermal Profile tab • 50°C for 5 minutes hold • 95°C for 2 minutes hold • 40 cycles of: 95°C, 3 seconds 60°C, 30 seconds

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, then add the unique reaction components (e.g., template).

   Note: Preparation of a master mix is crucial in qRT–PCR to reduce pipetting errors.

TaqMan™ probes reaction mix	
Component	**Volume for one 50-µL reaction**
SuperScript™ III RT/Platinum™ *Taq* Mix	1 µL[1]
2X Reaction Mix	25 µL
Forward primer, 10 µM	1 µL
Reverse primer, 10 µM	1 µL
Fluorogenic probe, 10 µM	0.5 µL
ROX™ Reference Dye (optional)	1 µL/0.1 µL[2]
RNaseOUT™ (optional)	1 µL
Template (1 pg to 1 µg total RNA)	≤10 µL
DEPC-treated water	to 50 µL

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

[2] See "Guidelines for ROX™ Reference Dye" 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.

Troubleshooting

Observation	Possible cause	Recommended action
No amplification product; relative fluorescent signal is equal to or smaller than 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.

Ordering information

The following products are also available. Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Amount	Source
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	5,000 units	10777-019
DNase I, Amplification Grade	100 units	18068-015

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Revision history: Pub. No. MAN0001006

Revision	Date	Description
C.0	3 May 2016	Removed LUX™ Primers and added additional instruments Format, style, and legal updates
B.0	16 February 2015	Baseline for this revision history

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