SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR

Catalog Numbers 11752-050 and 11752-250

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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 First-Strand Synthesis SuperMix for qRT–PCR provides the high-temperature capability of SuperScript™ III Reverse Transcriptase in an optimized SuperMix format for the synthesis of first-strand cDNA for use in real-time quantitative RT-PCR (qRT-PCR).

SuperScript™ III Reverse Transcriptase, included in the RT Enzyme Mix, is a version of M–MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme can be used to synthesize cDNA at a temperature range of 42–60°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Because SuperScript™ III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

RNaseOUT™ Recombinant Ribonuclease Inhibitor, also included in the enzyme mix, is an RNase inhibitor protein that safeguards against the degradation of target RNA due to ribonuclease contamination.

The 2X RT Reaction Mix includes oligo$(dT)_{20}$, random hexamers, $MgCl_2$, and dNTPs in a buffer formulation that has been optimized for qRT–PCR.

E. coli RNase H is provided as a separate tube in the kit to remove the RNA template from the cDNA:RNA hybrid molecule after first-strand synthesis. This has been shown to increase sensitivity in qRT-PCR.

This SuperMix formulation can be used to quantify fewer than 10 copies of a target gene in qRT–PCR, with a broad dynamic range that supports accurate quantification of high-copy mRNA from up to 1 µg of total RNA. Reagents are provided for 50 or 250 RT reactions of 20 µL each.

Contents and storage

Contents	Cat. No. 11752-050 (50 reactions)	Cat. No. 11752-250 (250 reactions)	Storage
RT Enzyme Mix (includes SuperScript™ III RT and RNaseOUT™)	100 µL	500 µL	Store components at –20°C. Stability can be extended by storing at –80°C.
2X RT Reaction Mix (includes oligo$(dT)_{20}$ (2.5 µM), random hexamers (2.5 ng/µL), 10 mM $MgCl_2$, and dNTPs)	500 µL	2 × 1.25 mL	
E. coli RNase H	50 µL	250 µL	

Guidelines for RNA

- High-quality, intact RNA is essential for full-length, high-quality cDNA synthesis and accurate mRNA quantification. Starting material can range up to 1 µg of total RNA.
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained. RNaseOUT™ is included in the RT Enzyme Mix to safeguard against degradation of target RNA due to ribonuclease contamination.
- To isolate total RNA, we recommend TRIzol™ Reagent (see "Ordering information" on page 2). Isolation of mRNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.
- We recommend using DNase I, Amplification Grade, to eliminate genomic DNA contamination from the total RNA (see "Ordering information" on page 2).

Synthesize first-strand cDNA

The following protocol has been optimized for generating first-strand cDNA for use in two-step qRT–PCR. Note that an incubation temperature of 50°C for 30 minutes is recommended as a general starting point. Higher temperatures (up to 60°C) may be used for difficult templates.

1. Combine the following kit components in a tube on ice. For multiple reactions, a master mix without RNA may be prepared:

Component	Amount
2X RT Reaction Mix	10 µL
RT Enzyme Mix	2 µL
RNA (up to 1 µg)	x µL
DEPC-treated water	to 20 µL

2. Gently mix tube contents and incubate at 25°C for 10 minutes.
3. Incubate tube at 50°C for 30 minutes.
4. Terminate the reaction at 85°C at 5 minutes, then chill on ice.
5. Add 1 µL (2 U) of *E. coli* RNase H and incubate at 37°C for 20 minutes.
6. Use diluted or undiluted cDNA in qPCR, or store at –20°C until use.

Note: Up to 10% of the qPCR reaction volume can be undiluted cDNA (e.g., for a 50-µL qPCR, use up to 5 µL of undiluted cDNA from step 6).

Troubleshooting

Observation	Possible cause	Recommended action
No qRT–PCR amplification product. Relative fluorescent signal less than or equal to 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 or degraded	Replace RNA if necessary.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
Poor sensitivity	Not enough starting template RNA	Increase the concentration of template RNA; use up to 1 µg of total RNA.
Product detected at higher than expected cycle number	RNA has been damaged or 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.
Product detected at lower than expected cycle number, and/or positive signal from no-template controls	Template or carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-qPCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipette tips or positive displacement pipettors.
	Too much sample added to reactions	Decrease the concentration of cDNA.
Unexpected bands after electrophoresis	RNA contamination with genomic DNA	Pre-treat RNA with DNase I.

Ordering information

The following qPCR kits are compatible with this kit and are available separately. Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Amount	Source
TRIzol™ Reagent	100 mL	15596-026
	200 mL	15596-018
Platinum™ Quantitative PCR SuperMix-UDG	100 reactions	11730-017
	500 reactions	11730-025
Platinum™ Quantitative PCR SuperMix-UDG with ROX™ dye	100 reactions	11743-100
	500 reactions	11743-500
SYBR™ GreenER™ qPCR SuperMix, Universal	100 reactions	11762-100
	500 reactions	11762-500
SYBR™ GreenER™ qPCR SuperMix	100 reactions	11760-100
	500 reactions	11760-500
SYBR™ GreenER™ qPCR SuperMix for iCycler™	100 reactions	11761-100
	500 reactions	11761-500
DNase I, Amplification Grade	100 reactions	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**.

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