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

Cat. No. 11752-050
11752-250

Size: 50 reactions
250 reactions

Store at –20°C

Description

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 (1, 2). The enzyme can be used to synthesize cDNA at a temperature range of 42–60ºC, 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 of the RNA preparation.

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.

Kit Components

Component	50-rxn kit	250-rxn kit
RT Enzyme Mix (includes SuperScript™ III RT and RNaseOUT™)	100 µl	500 µl
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

Two-Step qRT-PCR Kits

This kit is also included in the following two-step qRT-PCR kits:

- SuperScript™ Platinum® Two-Step qRT-PCR Kit with ROX, catalog nos. 11747-100 and 11747-500.
- SuperScript™ Platinum® SYBR® Green Two-Step qRT-PCR Kit with ROX, catalog nos. 11748-100 and 11748-500.
- SYBR® Greener™ Two-Step qRT-PCR Kit for ABI PRISM®, catalog nos. 11763-100 and 11763-500
- SYBR® Greener™ Two-Step qRT-PCR Kit for iCycler®, catalog nos. 11764-100 and 11764-500
- SYBR® Greener™ Two-Step qRT-PCR Kit, Universal, catalog nos. 11765-100 and 11765-500

Storage

Store components at –20ºC. Stability can be extended by storing at –80ºC.

Part no. 11752.pps

Rev. date: 15 Mar 2006

RNA Guidelines

- 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 the PureLink™ Micro-to-Midi™ Total RNA Purification System, TRIzol® Reagent, or the PureLink™ 96 Total RNA Purification Kit for high-throughput applications (see **Additional Products** on page 3). 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 **Additional Products** on page 3).

Protocol for First-Strand cDNA Synthesis

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

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

   Note: Up to 10% of the qPCR reaction volume may be undiluted cDNA (*e.g.*, for a 50-µl qPCR, use up to 5 µl of undiluted cDNA from Step 6 above).

Two-Step qRT-PCR Reactions

This kit is included with the two-step qRT-PCR kits listed on page 1. See the quick reference card provided with these kits, as well as the product documentation provided separately with the qPCR component of each kit, for qPCR guidelines and protocols.

This kit may also be used with stand-alone qPCR kits (see **Additional Products**, page 3).

Quality Control

The product was tested functionally in a two-step qRT-PCR procedure using total HeLa RNA as template. Kinetic analysis demonstrated a linear dose response with decreasing target concentration and detection of β-actin mRNA in 1 pg of total HeLa RNA.

Troubleshooting Guide

Use the following guide to identify and solve problems with the cDNA synthesis reaction in two-step qRT-PCR. Consult your qPCR manual for troubleshooting information on the qPCR component of the reaction.

Problem	Possible Cause	Probable Solution
No qRT-PCR 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 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.

Additional Products

The following qPCR kits are compatible with this kit and are available separately from Invitrogen.

	Amount	Cat. No.
PureLink™ Micro-to-Midi Total RNA Purification System	50 rxns	12183-018
PureLink™ 96 Total RNA Purification Kit	384 rxns	12173-011
TRIzol® Reagent	100 ml	15596-026
	200 ml	15596-018
Platinum® Quantitative PCR SuperMix-UDG	100 rxns	11730-017
	500 rxns	11730-025
Platinum® Quantitative PCR SuperMix-UDG with ROX	100 rxns	11743-100
	500 rxns	11743-500
SYBR Greener™ qPCR SuperMix, Universal	100 rxns	11762-100
	500 rxns	11762-500
SYBR Greener™ qPCR SuperMix for ABI PRISM®	100 rxns	11760-100
	500 rxns	11760-500
SYBR Greener™ qPCR SuperMix for iCycler®	100 rxns	11761-100
	500 rxns	11761-500
DNase I, Amplification Grade	100 units	18068-015

References

1. Kotewicz, M.L., D'Alessio, J.M., Driftmier, K.M., Blodgett, K.P., and Gerard, G.F. (1985) Cloning and overexpression of Moloney murine leukemia virus reverse transcriptase in *Escherichia coli*. *Gene 35,* 249.

2. Gerard, G.F., D'Alessio, J.M., Kotewicz, M.L., and Noon, M.C. (1986) Influence on stability in *Escherichia coli* of the carboxy-terminal structure of cloned Moloney murine leukemia virus reverse transcriptase. *DNA 5*, 271.

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