SuperScript™ VILO™ MasterMix

Catalog Number 11755-050, 11755-250, and 11755-500

Doc. Part No. 100012386 **Pub. No.** MAN0004286 **Rev.** A.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

Invitrogen™ SuperScript™ VILO™ MasterMix provides the high-temperature capability of SuperScript™ III Reverse Transcriptase (RT) in an optimized format for generating first-strand cDNA for use in real-time quantitative RT–PCR (qRT–PCR). This formulation can be used with very low and very high amounts of input RNA (up to 2.5 µg total RNA in a 20-µL reaction).

SuperScript™ VILO™ Master Mix includes SuperScript™ III RT, RNaseOUT™ Recombinant Ribonuclease Inhibitor, a proprietary helper protein, random primers, $MgCl_2$, and dNTPs.

SuperScript™ III RT is an engineered version of M–MLV RT with reduced RNase H activity and increased thermal stability. The enzyme can be used to synthesize cDNA at a temperature range of 42–55°C. 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 safeguards against the degradation of target RNA due to ribonuclease contamination.

Contents and storage

Contents	Cat. no. 11755-050 (50 reactions)	Cat. no. 11755-250 (250 reactions)	Cat. no. 11755-500 (500 reactions)	Storage conditions
SuperScript™ VILO™ Master Mix	200 µL	1000 µL	2000 µL	Store at –20°C (non-frost-free)

Important guidelines

cDNA synthesis guidelines

- High-quality, intact RNA is essential for accurate quantification in qPCR. RNA should be devoid of RNase contamination and aseptic conditions should be maintained. RNA quality can be analyzed using a bioanalyzer or by agarose gel electrophoresis.
- Starting material can range up to 2.5 µg of total RNA in a 20–µL cDNA synthesis reaction. RNA quantity can be determined using UV absorbance at 260 nm or the Qubit™ RNA Assay Kit and Qubit™ 2.0 Fluorometer (see "Related products" on page 4 for ordering information).
- To isolate total RNA, we recommend TRIzol™ Reagent, the PureLink™ RNA Mini Kit, or the MagMAX™-96 Total RNA Isolation Kit (see "Related products" on page 4). Isolation of mRNA is typically not necessary, although incorporating this step may improve the yield of specific cDNAs.
- DNase I, Amplification Grade, may be used to eliminate genomic DNA contamination from the total RNA (see "Related products" on page 4).
- Shorter incubation times and/or higher temperatures may be used (e.g., 50°C for 30 minutes), but may result in reduced yields of cDNA.
- For increased yields of cDNA, longer incubation times may be used (up to 120 minutes at 42°C).

qPCR using fluorescent primers or probes

Up to 10% of the qPCR reaction volume may be undiluted cDNA (e.g., for a 20-µL qPCR, use up to 2 µL of undiluted cDNA).

qPCR using SYBR™ Green or SYBR™ GreenER™ reagent

If you started with ≤100 ng of total RNA, up to 10% of the qPCR reaction volume may be undiluted cDNA (e.g., for a 20-µL qPCR, use up to 2 µL of undiluted cDNA).

If you started with >100 ng total RNA, we recommend testing a serial dilution of cDNA in qPCR for optimal results. Higher concentrations of cDNA may affect the signal baseline in SYBR™ Green and SYBR™ GreenER™ reactions.

Methods

Synthesize first-strand cDNA

The following protocol has been optimized for generating first-strand cDNA for use in two-step qRT–PCR. The reaction volume may be scaled as needed up to 100 µL. A negative RT control protocol is provided below.

1. For a single reaction, combine the following components in a sterile PCR tube or plate well on ice.

Component	Volume
SuperScript™ VILO™ MasterMix	4 µL
RNA (up to 2.5 µg)	X µL
DEPC-treated water	to 20 µL

2. Gently mix and incubate at 25°C for 10 minutes.
3. Incubate at 42°C for 60 minutes.
4. Terminate the reaction at 85°C at 5 minutes.
5. Use the diluted or undiluted cDNA in qPCR or store at –20°C.

Prepare negative RT control

1. For a volume of RNA = X µL, add the following to a sterile PCR tube or plate well on ice.

Component	Volume
SuperScript™ VILO™ MasterMix	4 µL
DEPC-treated water	16 – X µL

2. Incubate at 65°C for 10 minutes to denature the reverse transcriptase.
3. Add X µL of RNA (up to 2.5 µg) for a total reaction volume of 20 µL.
4. Proceed with steps 2–5 from “Synthesize first-strand cDNA” on page 3.

Related products

Product	Amount	Cat. no.
TRIzol™ Reagent	100 mL	15596-026
	200 mL	15596-018
PureLink™ RNA Mini Kit	10 preps	12183020
	50 preps	12183018A
MagMAX™-96 Total RNA Isolation Kit	96 rxns	AM1830
Qubit™ RNA Assay Kit	500 assays	Q32855
Qubit™ 2.0 Fluorometer	1 unit	Q32866
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**.

The information in this guide is subject to change without notice.

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