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SuperScript® VILO™ MasterMix

Part no. 100012386 MAN0004286 **Rev. Date:** 6 June 2011

Cat. nos:	Size:
11755-050	50 reactions
11755-250	250 reactions
11755-500	500 reactions

Store at –20°C (non-frost-free)

Description

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, MgCl2, 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.

Component	50 rxn kit	250 rxn kit	500 rxn kit
SuperScript® VILO™ MasterMix	200 µL	1000 µL	2000 µL

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Guidelines for cDNA Synthesis

- 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 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 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 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.

First-Strand cDNA Synthesis

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.

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 the "First-Strand cDNA Synthesis" protocol above.

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Additional Products

	Amount	Catalog number
TRIzol® Reagent	100 ml 200 ml	15596-026 15596-018
PureLink® RNA Mini Kit	10 preps 50 preps	12183020 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

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