SuperScript™ VILO™ cDNA Synthesis Kit

Catalog Number 11754-050 and 11754-250

Doc. Part No. 100002284 **Pub. No.** MAN0000749 **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

The Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit provides the high temperature capability of SuperScript™ III Reverse Transcriptase in an optimized format for generating first-strand cDNA for use in real-time quantitative RT–PCR (qRT–PCR). This formulation provides enhanced cDNA synthesis efficiency and 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), giving a linear response in message abundance as measured by qPCR.

The 10X SuperScript™ Enzyme Mix includes SuperScript™ III RT, RNaseOUT™ Recombinant Ribonuclease Inhibitor, and a proprietary helper protein. 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 42–60°C. Because SuperScript™ III RT is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA. RNaseOUT™ Recombinant Ribonuclease Inhibitor safeguards against the degradation of target RNA due to ribonuclease contamination.

The 5X VILO™ Reaction Mix includes random primers, $MgCl_2$, and dNTPs in a buffer formulation that has been optimized for qRT–PCR.

Note: This kit is included with EXPRESS Two-Step qRT–PCR kits. See that manual for complete qRT–PCR protocols and guidelines.

Reagents are provided for 50 or 250 20-µL cDNA synthesis reactions.

Contents and storage

Contents	Cat. no. 11754-050 (50 20-µL reactions)	Cat. no. 11754-250 (250 20-µL reactions)	Storage conditions
10X SuperScript™ Enzyme Mix	100 µL	500 µL	Store at –20°C (non-frost-free)
5X VILO™ Reaction Mix	200 µL	1000 µL	

Important 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. Note that for downstream qPCR using SYBR™ Green or SYBR™ GreenER™ reagent, you will need to dilute the cDNA generated from total RNA quantities above 100 ng (see "qPCR using SYBR™ Green or SYBR™ GreenER™ reagent" on page 3). RNA quantity can be determined using UV absorbance at 260 nm or the Quant-iT™ RNA Assay Kit (see "Related products" on page 3 for ordering information).
- 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 (see "Related products" on page 3). 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 3).
- 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 diluting the cDNA prior to qPCR, because higher concentrations of cDNA will affect the signal baseline in SYBR™ Green and SYBR™ GreenER™ reactions. For example, if you started with 2 µg of total RNA, prepare a 20-fold dilution of the resulting cDNA to achieve the concentration equivalent of starting with 100 ng of RNA. Then use up to 2 µL of the diluted cDNA in a 20-µL qPCR (≤10% of qPCR volume).

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.

1. For a single reaction, combine the following components in a tube on ice. For multiple reactions, prepare a master mix without RNA.

Component	Quantity
5X VILO™ Reaction Mix	4 µL
10X SuperScript™ Enzyme Mix	2 µL
RNA (up to 2.5 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 42°C for 60 minutes.
4. Terminate the reaction at 85°C at 5 minutes.
5. Use diluted or undiluted cDNA in qPCR (see "qPCR using fluorescent primers or probes" on page 2 and "qPCR using SYBR™ Green or SYBR™ GreenER™ reagent" on page 3), or store at –20°C until use.

Related products

Product	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
DNase I, Amplification Grade	100 units	18068-015

Limited product warranty

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