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# SuperScript® VILO™ cDNA Synthesis Kit

**Cat. no. 11754-010 Size: 10 reactions (20 µl/reaction)**

**Store at -20°C (non-frost-free)**

This sample kit provides reagents for 10 20-µl cDNA synthesis reactions. Kit sizes of 50 and 250 reactions are available. See page 4.

## Description

The 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, which can be used to synthesize cDNA at a temperature range of 42–60°C (1, 2). 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:** See the EXPRESS™ Two-Step qRT-PCR kit manual for complete qRT-PCR protocols using the SuperScript® VILO™ cDNA Synthesis Kit.

| Component | 10-rxn kit |
|---|---|
| 10X SuperScript® Enzyme Mix | 20 µl |
| 5X VILO™ Reaction Mix | 40 µl |

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## 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™, you will need to dilute the cDNA generated from total RNA quantities above 100 ng (see next page). RNA quantity can be determined using UV absorbance at 260 nm or the Quant-iT™ RNA Assay Kit (see page 4 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 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).

## 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.

## Protocol for 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.

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

| | |
|---|---|
| 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 below), or store at –20°C until use.

## 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™

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

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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).

## Additional Products

The following products are available on our website at www.invitrogen.com or by contacting technical support.

| | Cat. No. | Amount |
|---|---|---|
| Platinum® *Taq* DNA Polymerase | 10966-018 | 100 Reactions |
| | 10966-026 | 250 Reactions |
| | 10966-034 | 500 Reactions |
| | 10966-083 | 5,000 Reactions |

## References


1. Chou, Q., *et al*. (1992) *Nucl. Acids Res. 20*, 1717.
2. Sharkey, D.J., *et al*. (1994) *BioTechnology 12*, 506.
3. Westfall, B.A., *et al*. (1997) *Focus® 19.3*, 46.


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