SuperScript™ IV VILO™ Master Mix

invitrogen

USER GUIDE Pub. No. MAN0015862 Rev. B.0

Print options

Package contents

Catalog No.	Size
11766050	50 reaction (with ezDNase enzyme)
11766500	500 reaction (with ezDNase enzyme)
11756050	50 reaction
11756500	500 reaction

Kit contents

Storage conditions

Store all contents at –20°C (non-frost-free)

Required materials

- Template: RNA

Timing

- Preparation time: 5 minutes
- Total incubation time: 25–27 minutes

Product description

- The Invitrogen™ SuperScript™ IV VILO™ Master Mix with ezDNase enzyme is an optimized solution for first strand cDNA synthesis in two-step RT-PCR applications.
- The 5X master mix includes SuperScript™ IV Reverse Transcriptase, a proprietary recombinant RNase inhibitor, helper proteins, stabilizer proteins, oligo (dT)18, random hexamer primers, $MgCl_2$, and dNTPs.
- The SuperScript™ IV VILO™ No RT Control contains all the components of the SuperScript™ IV VILO™ Master Mix except the reverse transcriptase enzyme. The No RT Control is used in a RT minus reaction to verify the absence of genomic DNA (gDNA) contamination in the RNA sample.
- ezDNase enzyme (Cat. No. 11766051) is a novel double-strand specific thermolabile DNase that is used to remove gDNA contamination from template RNA prior to the RT reaction. The enzyme is available as part of the kit or as a standalone product.

Online resources

Visit our **product page** for additional information and protocols.
For support, visit **thermofisher.com/support**.

Guidelines for RNA preparation

- Use high-quality, intact RNA for accurate quantification. RNA must be devoid of RNase contamination and handled using aseptic conditions.
- Isolate total RNA with **TRIzol™ Reagent**, the **PureLink™ RNA Mini Kit**, or the **MagMAX™-96 Total RNA Isolation Kit**.
- Determine RNA quality using a bioanalyzer or by agarose gel electrophoresis.

Guidelines for reverse transcription

- Use up to 2.5 µg of total RNA as starting material in a 20-µL reaction.
- For GC-rich or structurally complex RNA templates, increasing the RT incubation temperature up to 65°C may improve cDNA synthesis results.
- To verify the absence of gDNA contamination in the template RNA, perform a no RT control reaction by substituting SuperScript™ IV VILO™ Master Mix with the SuperScript™ IV VILO™ No RT Control.

Limited product warranty

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If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

Reverse transcription protocol for SuperScript™ IV VILO™ Master Mix with ezDNase enzyme

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Prepare gDNA digestion reaction mix (on ice)</td><td>For each RT reaction or No RT Control reaction, prepare a 10 µL gDNA digestion reaction mix in a RNase-free tube on ice with the following components:
<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>10X ezDNase Buffer</td><td>1 µL</td></tr>
<tr><td>ezDNase enzyme</td><td>1 µL</td></tr>
<tr><td>Template RNA (1 pg to 2.5 µg total RNA)</td><td>varies</td></tr>
<tr><td>Nuclease-free Water</td><td>to 10 µL</td></tr>
</table></td></tr>
<tr><td>2  </td><td>Digest gDNA</td><td>Gently mix and incubate at 37°C for 2 minutes. Briefly centrifuge the reaction and place on ice.</td></tr>
<tr><td>3  </td><td>Prepare RT and No RT Control reaction mixes (on ice)</td><td>Add the following components to the tube containing the 10-µL reaction mix from step 2 on ice.
<table>
<tr><th>Component</th><th>RT reaction</th><th>No RT Control reaction</th></tr>
<tr><td>SuperScript™ IV VILO™ Master Mix</td><td>4 µL</td><td>—</td></tr>
<tr><td>SuperScript™ IV VILO™ No RT Control</td><td>—</td><td>4 µL</td></tr>
<tr><td>Nuclease-free Water</td><td>6 µL</td><td>6 µL</td></tr>
</table></td></tr>
<tr><td>4  </td><td>Anneal primers</td><td>Gently mix and incubate at 25°C for 10 minutes.</td></tr>
<tr><td>5  </td><td>Reverse transcribe RNA</td><td>Incubate at 50°C for 10 minutes.</td></tr>
<tr><td>6  </td><td>Inactivate enzyme</td><td>Incubate at 85°C for 5 minutes.</td></tr>
<tr><td>7  </td><td>qPCR amplification</td><td>Use the diluted or undiluted cDNA for qPCR or store at –20°C for up to one week, or –70°C for long term storage.
Click to see Guidelines for optimizing qPCR amplification.</td></tr>
</table>

Reverse transcription protocol for SuperScript™ IV VILO™ Master Mix (without ezDNase enzyme treatment)

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Prepare RT and No RT Control reaction mixes (on ice)</td><td>Add the following components to an empty RNase-free tube on ice.
<table>
<tr><th>Component</th><th>RT reaction</th><th>No RT Control reaction</th></tr>
<tr><td>SuperScript™ IV VILO™ Master Mix</td><td>4 µL</td><td>—</td></tr>
<tr><td>SuperScript™ IV VILO™ No RT Control</td><td>—</td><td>4 µL</td></tr>
<tr><td>Template RNA (1 pg to 2.5 µg total RNA)</td><td>varies</td><td>varies</td></tr>
<tr><td>Nuclease-free Water</td><td>to 20 µL</td><td>to 20 µL</td></tr>
</table></td></tr>
<tr><td>2  </td><td>Anneal primers</td><td>Gently mix and incubate at 25°C for 10 minutes.</td></tr>
<tr><td>3  </td><td>Reverse transcribe RNA</td><td>Incubate at 50°C for 10 minutes.</td></tr>
<tr><td>4  </td><td>Inactivate enzyme</td><td>Incubate at 85°C for 5 minutes.</td></tr>
<tr><td>5  </td><td>qPCR amplification</td><td>Use the diluted or undiluted cDNA for qPCR or store at –20°C for up to one week, or –70°C for long term storage.
Click to see Guidelines for optimizing qPCR amplification.</td></tr>
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