## Contents and storage

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

| SuperScript™ IV UniPrime™ One-Step RT-PCR System | | | |
|---|---|---|---|
| **Component** | **Cat. No. 12596025 (25 Rx [1])** | **Cat. No. 12596100 (100 Rx [1])** | **Cat. No. 12596500 (500 Rx [1])** |
| SuperScript™ IV RT Mix | 50 µL | 200 µL | 5 × Cat. No. 12596100 |
| 2X UniPrime™ RT-PCR Master Mix | 625 µL | 2 × 1.25 mL | |
| Water, Nuclease-free | 1.25 mL | 2 × 1.25 mL | |
| **SuperScript™ IV UniPrime™ One-Step RT-PCR System, Colored** | | | |
| **Component** | **Cat. No. 12597025 (25 Rx [1])** | **Cat. No. 12597100 (100 Rx [1])** | **Cat. No. 12597500 (500 Rx [1])** |
| SuperScript™ IV RT Mix, Red | 50 µL | 200 µL | 5 × Cat. No. 12597100 |
| 2X UniPrime™ RT-PCR Master Mix, Blue | 625 µL | 2 × 1.25 mL | |
| Water, Nuclease-free | 1.25 mL | 2 × 1.25 mL | |

[1] Number of reactions is based on 50 µL RT-PCR reaction volumes.

## Product description

The Invitrogen™ SuperScript™ UniPrime™ One-Step RT-PCR System is designed for sensitive end-point detection and analysis of RNA by RT-PCR. The convenient formulation enables both cDNA synthesis and PCR amplification to be performed in a single reaction tube using gene-specific primers.

- The system provides sensitivity down to 0.01 pg, can amplify RNA targets up to 13 kb in length, and is compatible with multiplex RT-PCR.
- A two-phase hot start activation mechanism allows easy room-temperature setup and benchtop stability of assembled reactions.
- The SuperScript™ IV RT Mix allows highly efficient reverse transcription step due to high thermostability, processivity, and ability to synthesize cDNA from variety of RNA samples.

## Online resources

- Visit the product page at thermofisher.com for additional information and protocols.
- For support, visit thermofisher.com/support.

## Product description, continued

- The 2X UniPrime™ RT-PCR Master Mix contains modified Platinum™ SuperFi™ II DNA Polymerase, which provides high specificity, high yields, and is ideally suited for PCR applications that require sequence accuracy. Novel master mix formulation enables universal primer annealing at 60°C for most primer pairs designed following the general design rules.
- A colored version of the kit makes it easy to track pipetting steps and enables direct sample loading on gels.

## Required materials

- Template RNA
- Gene specific primers (forward and reverse)

## Guidelines for RNA samples

- This kit is optimized for use with 0.01 pg to 1 µg of total RNA, and is compatible with total RNA, mRNA, viral RNA, and *in vitro* transcribed RNA.
- High-quality, intact RNA is essential for RT-PCR, particularly for long targets. 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 primer design

- Use gene-specific primers (GSPs) with the SuperScript™ IV UniPrime™ One-Step RT-PCR System. Oligo(dT) or random primers are not recommended, because nonspecific products can be generated, thereby reducing the amount of target RT-PCR product.
- A final concentration of 0.5 µM for each primer for single-plex reactions is recommended, but further optimization may be necessary.
- Design primers that anneal to the mRNA sequence in exons on both sides of an intron or exon/exon boundary, to allow differentiation between the amplified cDNA and potential contaminating genomic DNA, or perform gDNA removal on template RNA (See "**(*Optional*) Perform gDNA removal**").
- Ensure that primers are not self-complementary or complementary to each other at the 3' ends.

## Guidelines for cDNA synthesis and PCR

- Efficient cDNA synthesis can be accomplished by a 10-minute incubation at 50°C. This incubation is recommended as a general starting point.
- For GC-rich or structurally complex RNA templates, increasing the cDNA synthesis incubation temperatures up to 65°C may improve RT-PCR results.
- Use 30–40 cycles of amplification for RT-PCR products ≤3 kb, and up to 35 cycles for RT-PCR products >3 kb.
- For very low RNA input or rare targets, increasing the number of PCR cycles to 40 may improve results.
- The PCR extension time varies with the size of the amplicon. The recommended extension time is 30 seconds per 1 kb of amplicon (for a faster protocol, optimization of up to 20 seconds per 1 kb is possible).
- Prepared reactions can be stored at room temperature for up to 24 hours, when the target is ≤3 kb in length. For amplification of longer targets, preassembled reactions can be stored for up to 4 hours.
- In the colored format (Cat. No. 12597025, 12597100, 12597500), when the red SuperScript™ IV RT mix is added to the blue UniPrime™ RT-PCR Master Mix, the final reaction mix turns purple. Colored buffers allow the user to track pipetting steps, and also enable direct loading of RT-PCR products onto the gel.
- RT-PCR products obtained with both colored and colorless formats can be successfully used in further downstream applications such as sequencing, cloning, and genotyping.
- Certain components of the SuperScript™ IV UniPrime™ One-Step RT-PCR System may form precipitates after performing the RT-PCR reaction. These precipitates do not influence RT-PCR results or usability of the RT-PCR product for downstream purposes.

## Guidelines for multiplex RT-PCR

Due to its high processivity and specificity, as well as the specially formulated reaction buffer, SuperScript™ IV UniPrime™ One-Step RT-PCR System is suitable for multiplex RT-PCR applications without the need for significant optimization.

**Template**

- Recommended RNA range for detection is from 0.1 ng up to 1 µg.

**Primers**

- For multiplex RT-PCR, reduce primer concentration to 0.1 µM each. If required, primer concentration may be optimized in the range of 0.1–0.5 µM.
- Special attention to primer design parameters is critical for the success of multiplex RT-PCR.
- Optimal primers for multiplexing should be 21–34 nt in length and have 40–60% GC content. Ideally, G and C nucleotides should be distributed uniformly along the primer.
- Avoid significant homology between the primers and self-complementary primer regions, as well as three or more G or C nucleotides at the 3′ end.
- If possible, the primer should terminate with a G or C at the 3′ end.

**Amplicons**

- For product analysis on gel, up to 12 RNA targets can be amplified in a single reaction. Maximal amplicon length is up to 13 kb.
- For effective separation in agarose gel electrophoresis, ensure that the amplicon sizes differ sufficiently.

**Cycling**

- For amplification of multiple targets, an annealing time of 1 minute and an extension time of 30 seconds/kb at 72°C is recommended.
- In order to achieve more output, the extension time can be extended to 60 seconds/kb, for for targets up to 1 kb. For longer targets prolonged extension time is not recommended.
- 35 cycles for multiplex reaction is recommended.

## Prepare RT-PCR reaction mix

**Note**: If necessary, perform a gDNA digestion reaction with the template RNA before use in the RT-PCR reaction mix (See "**(*Optional*) Perform gDNA removal**" on page 5).

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Thaw reagents</td><td>Thaw reagents on ice. Mix and briefly centrifuge all reagents to ensure homogeneity before use.</td></tr>
<tr><td>2  </td><td>Prepare RT-PCR reaction mix</td><td>
a. Add the following components to each PCR tube.
<table>
<tr><th>Component</th><th>20-µL rxn</th><th>50-µL rxn</th><th>Final concentration</th></tr>
<tr><td>2X UniPrime™ RT-PCR Master Mix or<br>2X UniPrime™ RT-PCR Master Mix, Blue</td><td>10 µL</td><td>25 µL</td><td>1 X</td></tr>
<tr><td>Forward primer (10 µM)</td><td>1 µL</td><td>2.5 µL</td><td>0.5 µM</td></tr>
<tr><td>Reverse primer (10 µM)</td><td>1 µL</td><td>2.5 µL</td><td>0.5 µM</td></tr>
<tr><td>25X SuperScript™ IV RT Mix<br>25X SuperScript™ IV RT Mix, Red [1]</td><td>0.8 µL</td><td>2.0 µL</td><td>1 X</td></tr>
<tr><td>Template RNA</td><td>0.01 pg to 0.4 µg</td><td>0.01 pg to 1 µg</td><td>0.01 pg to 1 µg total RNA</td></tr>
<tr><td>Water, nuclease-free</td><td>Fill to 20 µL</td><td>Fill to 50 µL</td><td>—</td></tr>
</table>
[1] For no RT control reactions, add all components except the SuperScript™ IV RT Mix.<br>
b. Mix gently and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.<br>
Note: Assembled reactions can be stored for up to 24 h at room temperature prior amplification of targets up to 3 kb. For targets ≥3 kb, do not keep assembled reactions at room temperature for more than 4 h. With RNA samples of suboptimal purity, proceed immediately to thermal cycling.<br>
c. Proceed to "Perform RT-PCR and analyze results" on page 4.
</td></tr>
</table>

## Perform RT-PCR and analyze results

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Program thermal cycler</td><td>
a. Add the following components to each PCR tube. If preparing a master mix, multiply the volumes of components to all.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>No. cycles</th></tr>
<tr><td>Reverse transcription</td><td>50°C</td><td>10 minutes</td><td>1</td></tr>
<tr><td>RT inactivation/initial denaturation</td><td>98°C</td><td>2 minutes</td><td>1</td></tr>
<tr><td rowspan="3">Amplification</td><td>98°C</td><td>10 seconds</td><td rowspan="3">30–40 [1]</td></tr>
<tr><td>60°C</td><td>10 seconds</td></tr>
<tr><td>72°C</td><td>30 seconds/kb</td></tr>
<tr><td>Final extension</td><td>72°C</td><td>5 minutes</td><td>1</td></tr>
</table>
[1] Use up to 35 cycles for targets >3 kb.
</td></tr>
<tr><td>2  </td><td>Run thermal cycler</td><td>
a. Dispense the prepared reaction mix to separate PCR tubes, 8-well strips, or individual wells of a 96-well PCR plate.<br>
b. Place the reaction in the pre-heated thermal cycler and run program.
</td></tr>
<tr><td>3  </td><td>Analyze with gel electrophoresis</td><td>
a. Add gel loading buffer to PCR product, mix, and briefly centrifuge the contents.<br>
b. Reactions in the colored format do not need to be stained, load the purple sample directly into the gel well.<br>
Note: The blue and red tracking dyes that resolve on the gel correspond to PCR products of about 4 and 2 kb in length.<br>
c. For optimal separation using E-Gel™ precast agarose gels, dilute the sample 2- to 30-fold as directed in the recommendations for use of E-Gel™ agarose gels.<br>
d. After electrophoresis use your PCR product immediately in downstream applications, or store it at –20°C.
</td></tr>
</table>

## (Optional) Perform gDNA removal

If not using primers that allow differentiation between the amplified cDNA and potential contaminating gDNA, perform a gDNA digestion reaction with the template RNA. Use Invitrogen™ ezDNaze™ Enzyme for fast, easy, and RNA-friendly gDNA removal. Prepare a 10 µL gDNA digestion reaction mix for each RT-PCR reaction following the ezDNase™ Enzyme protocol.

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr>
<td>1<br>  </td>
<td>gDNA removal</td>
<td>
a. Mix the following components in a 0.2-mL, nuclease-free, thin-walled PCR tube on ice.
<table>
<tr><th>Component</th><th>10-µL rxn</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</td><td>0.01 pg to 1 µg total RNA</td></tr>
<tr><td>Water, nuclease-free</td><td>Fill to 10 µL</td></tr>
</table>
b. Gently mix and incubate at 37°C for 2 minutes.<br>
c. (Optional) If the RNA sample is to be used for RT-PCR of fragments ≥3 kb, incubate the sample at 55°C for 5 minutes in the presence of 10 mM DTT to inactivate the enzyme.<br>
d. Chill the tube on ice to bring the sample to room temperature, then briefly centrifuge and place the tube on ice.<br>
e. Proceed to"Prepare RT-PCR reaction mix" on page 3.<br>
Note: Depending on the RT-PCR reaction volume, use all or part of the prepared RNA volume after gDNA removal. Do not exceed 20% of final reaction volume (up to 10 µL per 50 µL RT-PCRreaction or up to 4 µL per 20 µL RT-PCR reaction).
</td>
</tr>
</table>

## Related products and equipment

| Product | Cat. No. |
|---|---|
| TRIzol™ Reagent | 15596026 |
| PureLink™ RNA Mini Kit | 12183020 |
| MagMAX™-96 Total RNA Isolation Kit | AM1830 |
| ezDNase™ Enzyme | 11766051 |
| RNaseZap™ RNase Decontamination Solution | AM9784 |
| RNaseZap™ RNase Decontamination Wipes | AM9786 |
| Water, nuclease-free | R0581 |
| E-Gel™ EX Agarose Gels, 1% | G401001 |
| E-Gel™ EX Agarose Gels, 2% | G401002 |
| E-Gel™ EX Double Comb Agarose Gels, 1% | A42345 |
| E-Gel™ EX Double Comb Agarose Gels, 2% | A42346 |
| E-Gel™ Power Snap Plus Electrophoresis Systems with Extended Warranty | G9301 |
| E-Gel™ Power Snap Plus Electrophoresis Systems, Starter Kit with Extended Warranty (E-Gel 48 1% with SYBR™ Safe) | G9341 |
| E-Gel™ Power Snap Plus Electrophoresis Systems, Starter Kit with Extended Warranty (E-Gel 48 2% with SYBR™ Safe) | G9342 |
| MicroAmp™ Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL | A30588 |
| EasyStrip™ Plus Tube Strip with Attached Ultra Clear Caps | AB2005 |
| ProFlex™ 96-well PCR System | 4484075 |
| ProFlex™ 3 × 32-well PCR System | 4484073 |
| PCR plastics | thermofisher.com/us/en/home/life-science/pcr/pcr-plastics/plastics-selection-guide.html |

## Limited product warranty

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17 February 2023