SuperScript™ IV Template-Switching RT Master Mix

Catalog Numbers A65423, A65424, A65425

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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™ IV Template-Switching RT Master Mix is designed for high template switching efficiency in cDNA synthesis reaction. It contains SuperScript™ IV Reverse Transcriptase, RNase Inhibitor, and all other reverse transcription reaction components in a convenient premixed format. Only RNA template, RT primer and template switching oligonucleotide (TSO) of choice need to be added.

To maximize template switching efficiency, the master mix contains proprietary template switching enhancer and leverages the terminal deoxynucleotidyl transferase (TdT) activity of SuperScript™ IV Reverse Transcriptase. When the reverse transcriptase reaches the 5' end of the RNA template, TdT activity adds 1–3 extra nucleotides to the cDNA end, enabling binding of template switching oligonucleotide and incorporation of known sequence at the 3' end of the resulting cDNA. Obtained cDNA can be used for amplification by PCR, gene expression analysis by real-time PCR, or as a template for 5'RACE or second strand cDNA synthesis.

The master mix is compatible with wide range of total RNA (from 2 pg to 4 µg) and is supplied with lysis buffer to enable direct cDNA synthesis from intact mammalian cells (1–1,000 cells per sample).

Contents and storage

Store all components at –20°C ± 5°C

Component	Cat. No. A65423 (48 reactions) [1]	Cat. No. A65424 (96 reactions) [1]	Cat. No. A65425 (480 reactions) [1]
10X Lysis Buffer	48 µL	96 µL	5 × Cat. No. A65424
4X SuperScript™ IV cDNA Synthesis Master Mix	240 µL	480 µL	
Nuclease-free water	1.25 mL	1.25 mL	

[1] Number of reactions is based on 20 µL reaction volumes.

Procedure guidelines

- Carefully read the entire contents of this user guide before starting the procedure.
- Keep all reagents, reaction mixes, and samples on ice, unless otherwise indicated.
- To prevent nuclease contamination:
  - Wear laboratory gloves during the procedures. Change gloves if you suspect that they are contaminated.
  - Use nucleic acid-free pipette tips to handle the reagents and avoid putting used tips into the reagent containers.
  - Decontaminate lab benches and pipettes before beginning the experiment.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- To prevent cross-contamination, carefully pipet reagents and samples to avoid splashing.
- Pipet viscous reagents slowly. Gently vortex or pipet up and down several times to ensure complete mixing.
- Prepare reactions in 0.2-mL PCR tubes (or plates) on ice or a benchtop tube cooler.
- For multiple reactions, prepare a single reaction mix, then dispense the appropriate volume to each reaction tube to minimize pipetting error. Scale common components proportionally based on the volumes indicated per sample, then add 5–10% overage.
- Room temperature is 20–25°C unless otherwise indicated.

Guidelines for RNA samples

- The kit is designed for use with intact mammalian cells or purified total RNA.
- For optimal results, use high-quality RNA.
- Ensure that input RNA is free of contaminants, such as residual traces of proteins, organic solvents, and salts that can degrade the RNA or decrease the activity and sensitivity of enzymes.
- Ensure that purified RNA is suspended in nuclease-free water or low-salt storage or elution buffer.
- RNA quantity in cells can vary by cell type, cell cycle, and cell health.
- For cell suspension and sorting, we recommend using balanced salt solutions, such as PBS, HBSS, EBSS, or other common PBS-based cell-sorting buffers.
- Recommended RNA input size varies from 2 pg to 4 µg.
  - Follow Protocol A when working with ≥10 ng input RNA (see page 2).
  - Follow Protocol B when working with intact cells or <10 ng RNA (see page 3).

Guidelines for primer design

- Use Capturing Oligo (dT) Primer (Cat. No. A65429), gene-specific primer (GSP) or another self-designed RT primer with the SuperScript™ IV Template-Switching RT Master Mix. A final concentration ≥ 4 µM for RT primer is recommended.
- Use Template Switching Oligo (Cat. No. A65427) or self-designed template switching oligonucleotide for template switching reaction. A final concentration ≥ 5.5 µM for template switching primer is recommended.
- If Capturing Oligo (dT) Primer and Template Switching Oligo are used for RT reactions, use Preamplification Primer (Cat. No. A65428) for amplification of obtained cDNA.
- If using self-designed primers, ensure that primers are not self-complementary or complementary to each other at the 3‘ends.

Before starting

- Thaw reagents at room temperature for 5 minutes, then mix thoroughly. Centrifuge the tubes and store on ice until use.
- Preheat a thermal cycler or thermal shaker to 72°C.
- Centrifuge all reagent tubes to collect the contents in the tube before opening.

Protocol A: For 10 ng to 4 µg of RNA

Prepare RT reaction mix

Combine the following components in a microcentrifuge tube, then briefly centrifuge.

Component	20 µL reaction [1]	Final concentration
Nuclease-free water	Fill to 20 µL	—
4X SuperScript™ IV cDNA Synthesis Master Mix	5 µL	1X
RT primer [2]	1 µL	≥ 4 µM
RNA sample	*x* µL	—
Template-switching oligonucleotide [2]	1 µL	≥ 5.5 µM

[1] Include 5–10% excess volume to compensate for pipetting error when preparing a master mix for multiple reactions.

[2] Refer to Guidelines for primer design (see page 2).

IMPORTANT! 4X SuperScript™ IV cDNA Synthesis Master Mix contains ribonuclease inhibitor. However, for samples that contain high amount of RNases, such as cells or other non-purified samples, additional amount of RNase Inhibitor might need to be added to the lysis mix (up to 0.8 U/µL). We recommend RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).

Perform the RT reaction

1. Set up the thermal cycling conditions for the reverse transcription reaction.

Step	Temperature	Heated lid temperature	Time
Reverse transcription and template switching	50°C	105°C	30 minutes [1]
Enzyme inactivation	85°C		5 minutes
Hold	4°C		Hold

[1] For cell samples, the incubation time can be increased up to 90 minutes to improve sensitivity. Specificity, however, may be lowered due to increased yields of intronic or other non-mRNA poly(A)-containing RNA.

2. Load the plate or tubes into the thermal cycler, then start the run.
3. After the run, briefly centrifuge the samples.
4. Store synthesized cDNA at -70°C for up to 1 week or use immediately in downstream application.

Protocol B: For low input RNA (<10 ng) and intact cells

The volumes described in this protocol are for a 20 µL reaction.

If necessary, prepare cells for lysis or dilute RNA to the desired concentration.

Note: With low input RNA (<10 ng), use of lysis buffer is recommended even when working with purified RNA.

Perform the lysis/hybridization reaction

1. Combine the following components in a microcentrifuge tube.

Component	10 µL mix [1]	Final concentration
10X Lysis buffer	1 µL	1X
RT primer [2]	1 µL	≥8.5 µM
Sample (cells or RNA)	*x* µL	—
Nuclease-free water	Fill to 10 µL	—

[1] Include 5–10% excess volume to compensate for pipetting error when preparing a master mix for multiple reactions.
[2] Refer to Guidelines for primer design (see page 2).

IMPORTANT! For samples that contain high amount of RNases, such as cells or other non-purified samples, additional amount of RNase Inhibitor might need to be added to the lysis mix (up to 0.8 U/µL). We recommend RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).

2. Pipet up and down 5–8 times to mix, then briefly centrifuge to collect the contents.

3. Incubate for 1 minute at 72°C, then briefly centrifuge to collect the contents. Immediately proceed to "Prepare RT reaction mix".

Prepare RT reaction mix

1. Combine the following components in a microcentrifuge tube.

Component	10 µL mix [1]	Final concentration
Nuclease-free water	4 µL	—
4X SuperScript™ IV cDNA Synthesis Master Mix	5 µL	1X
Template-switching oligonucleotide [2]	1 µL	≥ 5.5 µM

[1] Include 5–10% excess volume to compensate for pipetting error when preparing a master mix for multiple reactions.
[2] Refer to Guidelines for primer design (see page 2).

2. Add 10 µL of the RT reaction mix to 10 µL of the lysed/hybridized sample mix (from "Perform the lysis/hybridization reaction").

3. Pipet up and down 5–8 times to mix, then briefly centrifuge to collect the contents.

Perform the RT reaction

1. Set up the thermal cycling conditions for the reverse transcription reaction.

Step	Temperature	Heated lid temperature	Time
Reverse transcription and template switching	50°C	105°C	30 minutes [1]
Enzyme inactivation	85°C		5 minutes
Hold	4°C		Hold

[1] For cell samples, the incubation time can be increased up to 90 minutes to improve sensitivity. Specificity, however, may be lowered due to increased yields of intronic or other non-mRNA poly(A)-containing RNA.

2. Load the plate or tubes into the thermal cycler, then start the run.

3. After the run, briefly centrifuge the samples.

4. Store synthesized cDNA at -70°C for up to 1 week or use immediately in downstream application.

Recommendations for downstream applications

Note: Reagents for purification, preamplificatation, and second strand synthesis reagents are not supplied with the kit.

Preamplification of the cDNA

Preamplifcation of cDNA is optional to achieve necessary yield of cDNA product for further applications.

We recommend the use of Platinum™ SuperFi™ II PCR Master Mix (12368010). If Capturing Oligo (dT) Primer (Cat. No. A65429) and Template Switching Oligo (Cat. No. A65427) were used for the template switching reaction, use Preamplification Primer (Cat. No. A65428) for the preamplification of whole sample cDNA.

- Preamplifield cDNA can be analysed by qPCR, NGS or other applications.
- For NGS applications cleanup of preamplified cDNA is required to remove excess preamplification primers and enzymes that can impact NGS library preparation. We recommended using AMPure XP SPRI™ Reagent (Cat. No. A63880) according to manufacturer's recommendations.
- For qPCR applications, purification of preamplified cDNA is not required but can be used to concentrate samples for detection of lowexpression targets.
  - For purified cDNA samples—Proceed directly to qPCR with undiluted samples.
  - For unpurified cDNA samples—Dilute samples 1:10 to 1:100. The sample input volume should not exceed 20% of the final reaction volume.

Preamplification protocol using Platinum™ SuperFi™ II PCR Master Mix

1. Prepare preamplification reaction mix. Combine the following components for the required number of samples:

Component	100 µL reaction [1]	Final concentration
Nuclease-free water	29 µL	—
2X Platinum™ SuperFi™ II PCR Master Mix	50 µL	1X
Preamplification Primer Mix or self-designed primers	1 µL	0.5 µM
cDNA template	20 µL	—

[1] Include 5–10% excess volume to compensate for pipetting error when preparing a master mix for multiple reactions.

2. Pipet up and down or gently vortex to mix, then briefly centrifuge to collect the contents.

3. Set up the thermal cycling conditions for the preamplification reaction:

Step	Temperature	Time	Cycles
Initial denaturation	98°C	30 seconds	1
Denaturation	98°C	5-10 seconds	25-35
Annealing	60°C	30 seconds	
Extension	72°C	5-30 seconds	
Final extension	72°C	5 minutes	1
Hold	4°C	Hold	1

5’ RACE detection

- Purification of preamplified cDNA is not required but can be used to concentrate samples for detection of low-expression targets.
- Rapid Amplification of cDNA Ends (RACE) is a procedure for amplification of nucleic acid sequences from a messenger RNA template between a defined internal site and unknown sequences at either the 3' or the 5' -end of the mRNA.
- The 5′ RACE protocol entails a two-step process. Firstly, a template switching reverse transcription reaction is performed, yielding cDNAs with a known adapter sequence incorporated at 3’ end.
- In the second step, the 5′ end of the transcript is amplified utilizing gene specific primer and primer complementary to the adapter sequence. For PCR amplification we recommend using Platinum™ SuperFi™ II PCR Master Mix (Cat. No. 12368010) or Platinum™ SuperFi™ II Green PCR Master Mix (Cat. No. 12369010) according to the manufacturer's recommendations.

Second Strand cDNA Synthesis

Reverse transcription reaction with SuperScript™ IV Template-Switching RT Master Mix produces first strand cDNA. For second strand cDNA synthesis without intermediate organic extraction or ethanol precipitation steps, use the Invitrogen™ Second Strand cDNA Synthesis Kit (Cat. No. A48570).

qPCR

- Preamplifcation of cDNA is optional to achieve necessary yield of cDNA product for qPCR application.
- We recommend the use of TaqMan™ Fast Advanced Master Mix (Cat. No. 4444556) for all TaqMan™-based detection methods.
- We recommend the use of PowerTrack™ SYBR™ Green Master Mix (Cat. No. A46012) for all SYBR GREEN™-based detection methods.
- Do not exceed the sample input volume recommended by the manufacturer of the qPCR reagent.

NGS

- Preamplification of cDNA and purification of preamplified cDNA is required to achieve sufficient yield of cDNA product for NGS application. See “Preamplification of the cDNA” on page 4 or use a specialized preamplification kit such as the SuperScript™ IV Single Cell/Low-Input cDNA PreAmp Kit (Cat. No. 11752048).
- Both ligation and transposase based methods can be used for library preparation. Because the final preamplified product is dsDNA, use DNA library preparation kits for library preparation (e.g., Collibri™ ES DNA Library Prep Kits (Cat. No. A38605024) or Illumina® Nextera® XT DNA Library Preparation Kits).
- See the appropriate library preparation kit user guide for DNA input requirements and library preparation conditions.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Product	Catalog no.
Equipment	
Thermal cycler with heated lid, one of the following (or equivalent) • VeritiPro™ Thermal Cycler, 96-well • ProFlex™ 96-well PCR System • ProFlex™ 3 × 32-Well PCR System	 A48141 4484075 4484073
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Heat block and/or thermal mixer	MLS
Cooling rack for 0.2-mL PCR tubes or plates	MLS
Calibrated single-channel or multichannel pipettes (1 µL–1,000 µL)	MLS
Tubes, plates, and other consumables	
0.2-mL thin-walled PCR tubes or plates	thermofisher.com/plastics
Nuclease-free pipette tips	MLS
Disposable gloves	MLS
cDNA preamplification	
Capturing Oligo (dT) Primer	A65429
Template Switching Oligo	A65427
Preamplification Primer	A65428
Second Strand cDNA Synthesis Kit	A48570
Platinum™ SuperFi™ II PCR Master Mix	12368010
Platinum™ SuperFi™ II Green PCR Master Mix	12369010

Limited product warranty

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Revision	Date	Description
A.0	9 February 2024	New document for SuperScript™ IV Template-Switching RT Master Mix.