SuperScript™ IV CellsDirect™ cDNA Synthesis Kit
USER GUIDE

Catalog Numbers 11750150 and 11750350
Publication Number MAN0019059
Revision A.0
The information in this guide is subject to change without notice.

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:** Pub. No. MAN0019059

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.0</td>
<td>3 March 2020</td>
<td>New document for new product launch.</td>
</tr>
</tbody>
</table>

**Important Licensing Information:** These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**TRADEMARKS:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

©2020 Thermo Fisher Scientific Inc. All rights reserved.
## Contents

- **CHAPTER 1**  Product information .......................................................... 5  
  - Product description .................................................................................. 5  
  - Procedure overview ................................................................................ 6  
  - Contents and storage .............................................................................. 6  
  - Required materials not supplied ............................................................... 7  
  - Workflow .................................................................................................. 8  
- **CHAPTER 2**  Methods ........................................................................... 9  
  - Procedural guidelines ............................................................................... 9  
  - Before you begin ..................................................................................... 9  
  - Prepare cells for lysis ............................................................................. 10  
    - Prepare suspension or adherent cells for lysis ........................................ 10  
    - Prepare sorted cells for lysis ................................................................ 10  
  - Prepare the Lysis Solution on ice ......................................................... 11  
  - Perform cell lysis .................................................................................... 11  
  - Prepare the reverse transcription reactions .......................................... 12  
  - Perform reverse transcription .................................................................. 12  
  - Guidelines for qPCR or end-point PCR ............................................... 13  
- **APPENDIX A**  Troubleshooting .......................................................... 14  
- **APPENDIX B**  Perform the cell lysis procedure in a cell-culture plate .......................................................... 16  
  - Recommended volumes for lysis in a cell-culture plate .......................... 16  
  - Prepare cells for lysis in a cell-culture plate ........................................... 17  
  - Prepare the Lysis Solution ..................................................................... 17  
  - Perform cell lysis in a cell-culture plate ................................................. 18  
  - Prepare the reverse transcription reactions ........................................... 18  
- **APPENDIX C**  Supplemental information .......................................... 20  
  - Recommended volumes for high-density cell samples .......................... 20  
  - Good laboratory practices for PCR and RT-PCR ................................. 20
Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The SuperScript™ IV CellsDirect™ cDNA Synthesis Kit is optimized for the synthesis of first-strand cDNA directly from a mammalian cell lysate without first isolating RNA. Cell lysis and reverse transcription are performed in the same tube, and the resulting first-strand cDNA can be used directly in qPCR or PCR.

Key features of the kit:

• Compatible with a wide range of mammalian cell types grown under different treatment conditions.

• Single-tube format minimizes reagent loss, sample loss, and handling time.

• Entire lysate volume can be used in the first-strand cDNA synthesis reaction, providing greater yields with few cells, and allowing detection of rare transcripts.

• Generates high-quality cDNA for use in various applications, including qPCR and PCR.

• Simple protocol can be performed in under 1 hour.

• Compatible with TaqMan™ and SYBR GREEN™ qPCR master mixes.

• Optimized performance for cell numbers ranging from a single cell up to 10,000 cells per sample.

The SuperScript™ IV CellsDirect™ cDNA Synthesis Kit includes SuperScript™ IV Reverse Transcriptase. Features of this enzyme include:

• Reduced RNase H activity.

• High thermal stability, up to 65°C.

• High yields of cDNA in the first-strand synthesis reaction, allowing greater sensitivity and enhanced detection of rare transcripts.

• Superior tolerance to RT inhibitors from the cell lysates.
Procedure overview

In traditional reverse transcription (RT), RNA is first isolated from cells in a time-consuming procedure that can lead to loss of precious material. With the SuperScript™ IV CellsDirect™ cDNA Synthesis Kit, cells are lysed and cDNA is generated from the lysate in a single tube/well with minimal handling and no sample loss. DNase I treatment is used to eliminate genomic DNA prior to first-strand synthesis.

This document provides guidance for cell lysis and cDNA synthesis in a single tube. If you are performing cell lysis directly in a cell-culture plate, follow Appendix B, “Perform the cell lysis procedure in a cell-culture plate”.

Contents and storage

Table 1  SuperScript™ IV CellsDirect™ cDNA Synthesis Kit

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cat. No. 11750150 (50 reactions)</th>
<th>Cat. No. 11750350 (500 reactions)</th>
<th>Storage[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperScript™ IV CellsDirect™ Lysis Solution[^2]</td>
<td>2 x 1.25 mL</td>
<td>3 x 9 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>SuperScript™ IV CellsDirect™ Stop Solution[^2]</td>
<td>250 µL</td>
<td>2 x 1.25 mL</td>
<td></td>
</tr>
<tr>
<td>DNase I[^3]</td>
<td>55 µL</td>
<td>500 µL</td>
<td></td>
</tr>
<tr>
<td>Lysis Enhancer[^2]</td>
<td>25 µL</td>
<td>250 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>SuperScript™ IV RT Master Mix</td>
<td>400 µL</td>
<td>4 x 1 mL</td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV No RT Control</td>
<td>400 µL</td>
<td>4 x 1 mL</td>
<td></td>
</tr>
</tbody>
</table>

[^1]: See the expiration date on the label.
[^2]: These reagents can be ordered separately as Cat. No. 11750550.
[^3]: DNase I can be ordered separately as Cat. No. 18047019.
Required materials not supplied

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal cycler, one of the following (or equivalent):</td>
<td></td>
</tr>
<tr>
<td>• Veriti™ 96-Well Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>• SimpliAmp™ Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>• ProFlex™ PCR System</td>
<td></td>
</tr>
<tr>
<td>• MiniAmp™ Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>Contact your local sales office.</td>
<td></td>
</tr>
<tr>
<td>Benchtop microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Plate centrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Automated cell counter or hemocytometer</td>
<td>MLS</td>
</tr>
<tr>
<td><em>(Optional)</em> Orbital shaker⁴</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettors</td>
<td>MLS</td>
</tr>
<tr>
<td>Cold packs or ice</td>
<td>MLS</td>
</tr>
<tr>
<td>Ice bucket</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Tubes, plates, and other consumables</strong></td>
<td></td>
</tr>
<tr>
<td>Nuclease-free pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Nuclease-free microcentrifuge tubes</td>
<td>MLS</td>
</tr>
<tr>
<td>96-well PCR reaction plate or tubes</td>
<td>thermofisher.com/plastics</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td></td>
</tr>
<tr>
<td><em>(Optional)</em> Trypsin⁵</td>
<td>MLS</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>AM9938</td>
</tr>
<tr>
<td>PBS (1X), pH 7.4</td>
<td>AM9624</td>
</tr>
</tbody>
</table>

¹⁴ For use during the cell lysis procedure in cell-culture plates.
⁵ Required for detachment of adherent cells.
Workflow

Prepare cells for lysis

- a. Count the cells, wash in 4°C PBS, then resuspend in 4°C PBS.
- b. Transfer 5 μL aliquots of the cell suspension to the bottom of PCR tubes or plate wells.

* Keep the cells on cold packs or ice.

Prepare the Lysis Solution on ice

Add Lysis Enhancer and DNase I to SuperScript™ IV CellsDirect™ Lysis Solution.

* Keep the reagents on cold packs or ice.

Perform cell lysis

- a. Add the prepared Lysis Solution to each cell sample, then incubate for 7 minutes at room temperature.
- b. Add SuperScript™ IV CellsDirect™ Stop Solution to each cell sample, then incubate for 2 minutes at room temperature.

Prepare the reverse transcription reactions

Add SuperScript™ IV RT Master Mix to the lysate.

* Keep the lysates on cold packs or ice.

Perform reverse transcription

**STOPPING POINT** Single-stranded cDNA can be stored at −20°C for 1 week, or at −70°C for long-term storage.

Use the cDNA samples directly for qPCR or end-point PCR

See “Guidelines for qPCR or end-point PCR” on page 13.
Methods

Procedural guidelines

- Perform all procedures on cold packs or ice unless otherwise indicated.
- This procedure is optimized for a maximum of 10,000 cells per reaction. If needed, volumes can be adjusted for cell numbers greater than 10,000 (see “Recommended volumes for high-density cell samples” on page 20).

Note: Using too many cells per lysis reaction can result in incomplete lysis and/or inhibition of RT-PCR.

- If you are performing cell lysis in a cell-culture plate, follow Appendix B, “Perform the cell lysis procedure in a cell-culture plate”.
- Do not vortex the Lysis Solution or lysates as foam can develop. Assay performance is not affected if some foaming occurs.
- To prevent cross-contamination, carefully pipet reagents and samples, to avoid splashing.
- To prevent nuclease contamination:
  - Wear laboratory gloves during the procedures. Gloves protect you from the reagents, and they protect the nucleic acid from nucleases that are present on skin.
  - 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 you begin.
- Room temperature is 20–25°C unless otherwise indicated.
- Follow “Good laboratory practices for PCR and RT-PCR” on page 20.

Before you begin

- Thaw all lysis reagents, gently invert or finger-tap each tube to mix, then place on ice.
  Mix the SuperScript™ IV CellsDirect™ Lysis Solution gently (do not vortex) to avoid formation of foam.
- Centrifuge all reagent tubes before opening to collect the contents at the bottom of the tubes.
- Place PBS on ice and chill to 4°C.
Prepare cells for lysis

**Prepare suspension or adherent cells for lysis**

**IMPORTANT!** Always keep cells on cold packs or ice unless otherwise indicated.

Detach adherent cells using a trypsin-based dissociation method before starting this procedure.

**Note:** If you are performing cell lysis in a cell-culture plate, follow Appendix B, “Perform the cell lysis procedure in a cell-culture plate”.

1. *(Optional)* Count the cells using an automated cell counter, or hemocytometer.
2. Centrifuge the cell suspension at 300 × *g* for 5 minutes, or according to the cell line recommendations.
3. Aspirate and discard the medium, then place the cells on ice.
4. Wash the cells with 0.5 mL of 4°C PBS per 1 × 10⁶ cells, then centrifuge at 300 × *g* for 5 minutes, or according to the cell line recommendations.
5. Aspirate and discard the PBS without disturbing the pellet, then resuspend the cells with 0.5 mL of 4°C PBS per 1 × 10⁶ cells.
6. Count the cells using an automated cell counter, or hemocytometer. Ensure the cell density is 1–10,000 cells per 5 μL.
   If needed, adjust the cell density with 4°C PBS.
7. Transfer a 5 μL aliquot of each cell suspension to the bottom of a 0.2 mL thin-walled PCR tube or plate well, then place on ice.
   Ensure the cell suspension is transferred to the bottom (not the wall) of the tube or well.
8. Keep the cells on ice and immediately proceed to “Prepare the Lysis Solution on ice” on page 11.

**Prepare sorted cells for lysis**

1. Sort cells in PBS at a density of 1–10,000 cells per 5 μL volume in a 0.2 mL thin-walled PCR tube or plate well, then place the cells on ice.
2. Store sorted cells at −70°C or proceed to “Prepare the Lysis Solution on ice” on page 11.
Prepare the Lysis Solution on ice

**IMPORTANT!** Always keep the reagents on cold packs or ice unless otherwise indicated.

Calculate the number of samples. Scale the components proportionally based on the volume per sample, then add 10% overage.

1. Prepare the Lysis Solution by combining the following components, in the order indicated, according to the following table.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per sample</th>
<th>Volume for n samples[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperScript™ IV CellsDirect™ Lysis Solution</td>
<td>23.28 µL</td>
<td>n x 25.61 µL</td>
</tr>
<tr>
<td>Lysis Enhancer (100X)</td>
<td>0.24 µL</td>
<td>n x 0.26 µL</td>
</tr>
<tr>
<td>DNase I (50X)</td>
<td>0.48 µL</td>
<td>n x 0.53 µL</td>
</tr>
<tr>
<td><strong>Total volume per sample</strong></td>
<td><strong>24 µL</strong></td>
<td><strong>n x 26.4 µL</strong></td>
</tr>
</tbody>
</table>

[^1]: Includes 10% overage.

**Note:** Lysis Enhancer and DNase I are optional if you are using 1–100 cells per sample.

2. Invert the tube or pipet up and down 5–8 times to mix (do not vortex), then place the Lysis Solution on ice.
   Mix the Lysis Solution gently to avoid formation of foam.

3. To prevent loss of DNase I activity, immediately proceed to “Perform cell lysis” on page 11.

Perform cell lysis

1. Add 24 µL of the prepared Lysis Solution to each cell sample-containing tube or well (on ice). Ensure the Lysis Solution completely covers the cells.

2. Keep the cells on ice and gently pipet up and down 5–8 times to mix. Do not vortex.

3. Remove the cells from ice, then incubate the lysis reaction for at least 7 minutes at room temperature. Do not exceed 15 minutes.

4. Add 3 µL of SuperScript™ IV CellsDirect™ Stop Solution to each sample.
   **Note:** SuperScript™ IV CellsDirect™ Stop Solution is not required if cells were lysed without Lysis Enhancer and DNase I.

5. Gently pipet up and down 5–8 times to mix. Do not vortex.
6. Centrifuge briefly to bring the contents to the bottom of the tube or plate well.

7. Incubate for 2 minutes at room temperature.

   **Note:** If needed, the incubation time can be extended to 10 minutes without affecting the quality of the lysate.

8. Place the lysates on ice, then proceed to “Prepare the reverse transcription reactions” on page 12.

   *(Optional)* Store the lysates on ice for up to 1 hour.

### Prepare the reverse transcription reactions

1. Thoroughly vortex the SuperScript™ IV RT Master Mix and SuperScript™ IV No RT Control, then centrifuge briefly to bring the contents to the bottom of each tube.

2. Transfer 8 μL of SuperScript™ IV RT Master Mix to each lysate-containing tube or well on ice for a final reaction volume of 40 μL.

   **Note:** For lysate volumes <32 μL, adjust the volume with nuclease-free water.

   For the minus-RT control, add 8 μL of SuperScript™ IV No RT Control instead of SuperScript™ IV RT Master Mix.

3. Cap the tubes or cover the plates, then immediately proceed to “Perform reverse transcription” on page 12.

### Perform reverse transcription

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annealing</td>
<td>1</td>
<td>25°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Reverse transcription</td>
<td>2</td>
<td>50°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Enzyme inactivation</td>
<td>3</td>
<td>85°C</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Hold</td>
<td>4</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

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

3. At the end of the run, store the cDNA samples as indicated or proceed directly to qPCR or end-point PCR. See “Guidelines for qPCR or end-point PCR” on page 13.

   Store the single-stranded cDNA on ice for immediate use, at −20°C for up to 1 week, or at −70°C for long-term storage.
Guidelines for qPCR or end-point PCR

The cDNA generated using this kit can be used directly in PCR without additional purification.

<table>
<thead>
<tr>
<th>For...</th>
<th>Do this...</th>
</tr>
</thead>
<tbody>
<tr>
<td>qPCR</td>
<td>Prepare the PCR reactions as indicated.</td>
</tr>
<tr>
<td>• For SYBR GREEN™ Assays—Add the prepared cDNA sample at 10% of the PCR reaction volume, or follow the recommendations provided with the qPCR reagent.</td>
<td></td>
</tr>
<tr>
<td>• For TaqMan™ Assays—Add the prepared cDNA sample at 10–20% of the PCR reaction volume, or follow the recommendations provided with the qPCR reagent.</td>
<td></td>
</tr>
<tr>
<td>End-point PCR</td>
<td>Add the cDNA sample at ≤10% of the PCR reaction volume, or follow the recommendations provided with the reagent used.</td>
</tr>
</tbody>
</table>
## Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR product or low product yield</td>
<td>A pipetting error occurred.</td>
<td>Ensure all steps are performed as described in the procedure.</td>
</tr>
<tr>
<td></td>
<td>Too many cells were used in the lysis reaction.</td>
<td>Repeat the procedure using ≤10,000 cells per lysis reaction.</td>
</tr>
<tr>
<td></td>
<td>If too many cells per sample are used in the procedure, the RNase in the sample cannot be inactivated and/or cellular components or debris could inhibit reverse transcription or PCR.</td>
<td>Repeat the procedure, increasing the Lysis Solution volume (see “Recommended volumes for high-density cell samples” on page 20).</td>
</tr>
<tr>
<td>Excess cell-culture medium or PBS was present during the lysis reaction.</td>
<td>If &gt;5 μL of PBS remains in the samples when the Lysis Solution is added, the Lysis Solution is too dilute to inactivate cellular RNases. For adherent cells, remove as much PBS as possible before adding the Lysis Solution to the cells. For cells that are resuspended in PBS, ensure ≤5 μL of PBS is used for each sample containing 1–10,000 cells.</td>
<td>Add an RNA positive control in the lysis reaction.</td>
</tr>
<tr>
<td>Inhibitors were present in the RT reaction or PCR.</td>
<td>Repeat the procedure using fewer cells per lysis reaction.</td>
<td>Add an RNA positive control in the lysis reaction.</td>
</tr>
<tr>
<td>RNA was degraded before the procedure was started.</td>
<td>Keep the cells on ice before starting the cell lysis procedure. Remove the cells from ice only after the Lysis Solution is added.</td>
<td>Add an RNA positive control in the RT reaction.</td>
</tr>
<tr>
<td>The lysates were incubated too long at room temperature.</td>
<td>Do not allow the lysates to incubate longer than 15 minutes at room temperature after the SuperScript™ IV CellsDirect™ Stop Solution has been added. After the lysis reaction, store the lysates on ice for up to 1 hour.</td>
<td>Perform a pilot experiment to determine the optimal number of cells that are required for detection of the target gene.</td>
</tr>
<tr>
<td>The expression level of the target gene was too low to detect in a small number of cells.</td>
<td>Perform a pilot experiment to determine the optimal number of cells that are required for detection of the target gene.</td>
<td>Perform a control reaction with purified RNA.</td>
</tr>
<tr>
<td>There was a problem with the PCR primers.</td>
<td>Redesign the primers.</td>
<td>Redesign the primers.</td>
</tr>
<tr>
<td>Observation</td>
<td>Possible cause</td>
<td>Recommended action</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>PCR products in the minus-RT control reactions</td>
<td>Cross-contamination occurred during the procedure.</td>
<td>Follow “Good laboratory practices for PCR and RT-PCR” on page 20.</td>
</tr>
<tr>
<td></td>
<td>Lysis Enhancer and DNase I were not added to the Lysis Solution.</td>
<td>Prepare the Lysis Solution as described (see “Prepare the Lysis Solution on ice” on page 11).</td>
</tr>
<tr>
<td></td>
<td>Genomic DNA was not completely removed.</td>
<td>Repeat the cell lysis procedure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure the cell suspension is transferred to the bottom (not the wall) of the tube or well.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure DNase I is added to the Lysis Solution.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure the Lysis Solution completely covers the cells.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure the incubation is performed as described.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ensure the lysis reagents are kept on ice throughout the procedure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ensure the lysis reaction is performed at room temperature.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use exon-exon junction primers that are cDNA-specific and do not bind to genomic DNA.</td>
</tr>
</tbody>
</table>
Perform the cell lysis procedure in a cell-culture plate

The following procedure provides guidance to lyse cells in a cell-culture plate. After the cell lysis procedure, the lysate is transferred to a nuclease-free 96-well reaction plate or tube for the reverse transcription reaction.

Table 2 provides the recommended volumes of Lysis Solution and SuperScript™ IV CellsDirect™ Stop Solution for the procedure.

Recommended volumes for lysis in a cell-culture plate

Table 2  Recommended volumes: cell-culture plate format

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per well</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>384-well</td>
</tr>
<tr>
<td><strong>Lysis Solution</strong></td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV CellsDirect™ Lysis Solution</td>
<td>29.1 μL</td>
</tr>
<tr>
<td>Lysis Enhancer (100X)</td>
<td>0.3 μL</td>
</tr>
<tr>
<td>DNase I (50X)</td>
<td>0.6 μL</td>
</tr>
<tr>
<td><strong>Total volume per well</strong></td>
<td>30 μL</td>
</tr>
<tr>
<td><strong>Stop Solution</strong></td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV CellsDirect™ Stop Solution</td>
<td>3.0 μL</td>
</tr>
</tbody>
</table>

**Note:** For the reverse transcription reaction, combine 32 μL of each cell lysate with 8 μL of SuperScript™ IV RT Master Mix in a nuclease-free 96-well reaction plate or tube.
Prepare cells for lysis in a cell-culture plate

1. Seed cells at an appropriate density to obtain 1–10,000 cells per well at the time of harvest.

2. To harvest the cells, place the cell-culture plate on ice, then aspirate and discard the medium.

   **IMPORTANT!** Keep the cells on cold packs or ice for the remaining steps of this procedure.

3. Wash the cells with 4°C PBS.

4. Aspirate and discard the PBS without disturbing the cells.

   **IMPORTANT!** Remove as much PBS as possible from the wells.

5. Keep the cells on ice and immediately proceed to “Prepare the Lysis Solution” on page 17.

Prepare the Lysis Solution

**IMPORTANT!** Always keep the reagents on cold packs or ice unless otherwise indicated.

Calculate the number of samples. Scale the components proportionally based on the volume per sample, then add 10% overage.

1. Prepare the Lysis Solution by combining the following components, in the order indicated, according to Table 2.

   1. SuperScript™ IV CellsDirect™ Lysis Solution
   2. Lysis Enhancer
   3. DNase I

   **Note:** Lysis Enhancer and DNase I are optional if you are using 1–100 cells per sample.

2. Invert the tube or pipet up and down 5–8 times to mix (do not vortex), then place the Lysis Solution on ice.
   Mix the Lysis Solution gently to avoid formation of foam.

3. To prevent loss of DNase I activity, immediately proceed to “Perform cell lysis in a cell-culture plate” on page 18.
Perform cell lysis in a cell-culture plate

1. Add the prepared Lysis Solution to each cell sample-containing well (on ice), according to Table 2. Ensure the Lysis Solution completely covers the cells.

2. Keep the cells on ice and mix the samples using one of the following methods. Do not vortex.
   - **By pipetting**—Gently pipet up and down 5–8 times.
   - **Using a plate shaker**—Shake on an orbital shaker set on low speed.

3. Remove the cells from ice, then incubate the lysis reaction for at least 7 minutes at room temperature. Do not exceed 15 minutes.

4. Add SuperScript™ IV CellsDirect™ Stop Solution to each sample, according to Table 2.
   
   **Note:** SuperScript™ IV CellsDirect™ Stop Solution is not required if cells were lysed without Lysis Enhancer and DNase I.

5. Mix the samples according to your mixing method. Do not vortex.
   - **By pipetting**—Gently pipet up and down 5–8 times.
   - **Using a plate shaker**—Shake on an orbital shaker set on low speed.

6. Incubate for 2 minutes at room temperature.
   
   **Note:** If needed, the incubation time can be extended to 10 minutes without affecting the quality of the lysate.

7. Place the lysates on ice, then proceed to “Prepare the reverse transcription reactions” on page 18.

*(Optional)* Store the lysates on ice for up to 1 hour.

Prepare the reverse transcription reactions

1. Thoroughly vortex the SuperScript™ IV RT Master Mix and SuperScript™ IV No RT Control, then centrifuge briefly to bring the contents to the bottom of each tube.

2. Transfer 32 μL of each lysate to the appropriate wells of a nuclease-free 96-well reaction plate or tube on ice.

3. Add 8 μL of SuperScript™ IV RT Master Mix to each lysate-containing tube or well for a final reaction volume of 40 μL.
   
   **Note:** For lysate volumes <32 μL, adjust the volume with nuclease-free water.
For the minus-RT control, add 8 μL of SuperScript™ IV No RT Control instead of SuperScript™ IV RT Master Mix.

4. Cap the tubes or cover the plates, then immediately proceed to “Perform reverse transcription” on page 12.
Supplemental information

Recommended volumes for high-density cell samples

The following table provides the recommended reagent volumes to lyse, then perform reverse transcription with samples containing >10,000 cells.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per sample</th>
<th>1 x 10^5 cells</th>
<th>1 x 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lysis Solution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV CellsDirect™ Lysis Solution</td>
<td>48.5 μL</td>
<td>97.0 μL</td>
<td>194.0 μL</td>
</tr>
<tr>
<td>Lysis Enhancer (100X)</td>
<td>0.5 μL</td>
<td>1.0 μL</td>
<td>2.0 μL</td>
</tr>
<tr>
<td>DNase I (50X)</td>
<td>1.0 μL</td>
<td>2.0 μL</td>
<td>4.0 μL</td>
</tr>
<tr>
<td>Total volume per well</td>
<td>50 μL</td>
<td>100 μL</td>
<td>200 μL</td>
</tr>
<tr>
<td><strong>Stop Solution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV CellsDirect™ Stop Solution</td>
<td>5.0 μL</td>
<td>10.0 μL</td>
<td>20 μL</td>
</tr>
<tr>
<td><strong>Reverse transcription reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lysate</td>
<td></td>
<td></td>
<td>32.0 μL</td>
</tr>
<tr>
<td>SuperScript™ IV RT Master Mix</td>
<td></td>
<td></td>
<td>8.0 μL</td>
</tr>
<tr>
<td>Total reaction volume</td>
<td></td>
<td></td>
<td>40 μL</td>
</tr>
</tbody>
</table>

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
• Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
• Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.
WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d’utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
• Limiter l’inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu’avec une ventilation adéquate (par exemple, sorbonne).
• Vérifier régulièrement l’absence de fuite ou d’écoulement des produits chimiques. En cas de fuite ou d’écoulement d’un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
• Manipuler les déchets chimiques dans une sorbonne.
• Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
• Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
• Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
• Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
• IMPORTANT ! Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s’appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

Documentation and support

Related products

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperScript™ IV CellsDirect™ Lysis Reagent[^1]</td>
<td>11750550</td>
</tr>
<tr>
<td>DNase I</td>
<td>18047019</td>
</tr>
<tr>
<td>TaqMan™ Fast Advanced Master Mix</td>
<td>4444556</td>
</tr>
<tr>
<td>TaqMan™ Cells-to-CT™ Control Kit</td>
<td>4386995</td>
</tr>
<tr>
<td>PowerTrack™ SYBR™ Green Master Mix</td>
<td>A46012</td>
</tr>
<tr>
<td>Platinum™ SuperFi™ II Green PCR Master Mix</td>
<td>12369010</td>
</tr>
<tr>
<td>E-Gel™ Power Snap Electrophoresis System Starter Kit, EX 1%</td>
<td>G8341ST</td>
</tr>
<tr>
<td>E-Gel™ Power Snap Electrophoresis System Starter Kit, EX 2%</td>
<td>G8342ST</td>
</tr>
<tr>
<td>Mother E-Base™ Device (for high-throughput agarose gel analysis)</td>
<td>EBM03</td>
</tr>
<tr>
<td>E-Gel™ 96 Agarose Gels with SYBR™ Safe DNA Gel Stain, 1%</td>
<td>G720801</td>
</tr>
<tr>
<td>E-Gel™ 96 Gels with SYBR™ Safe DNA Gel Stain, 2%</td>
<td>G720802</td>
</tr>
</tbody>
</table>


Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
• Product documentation
  – User guides, manuals, and protocols
  – Certificates of Analysis
  – Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies’ General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.