SuperScript™ IV CellsDirect™ cDNA Synthesis Kit

Catalog Numbers 11750150 and 11750350

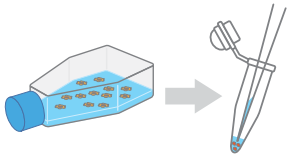
Pub. No. MAN0019141 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *SuperScript™ IV CellsDirect™ cDNA Synthesis Kit User Guide* (Pub. No. MAN0019059). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of the SuperScript™ IV CellsDirect™ cDNA Synthesis Kit. For detailed instructions, supplemental procedures, and troubleshooting, see the *SuperScript™ IV CellsDirect™ cDNA Synthesis Kit User Guide* (Pub. No. MAN0019059).

Cell lysis and reverse transcription procedure

1 Prepare cells for lysis



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

Note: If you are using sorted cells, sort the cells in PBS at a density of 1–10,000 cells per 5 µL volume in a PCR tube or plate well, then place the cells on ice.

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

a. *(Optional)* Count the cells using an automated cell counter, or hemocytometer.

b. Centrifuge the cell suspension at 300 × *g* for 5 minutes.

c. Aspirate and discard the medium, then place the cells on ice.

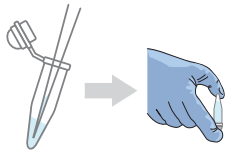
d. Wash the cells with 0.5 mL of 4°C PBS per 1 × 10^6 cells, then centrifuge at 300 × *g* for 5 minutes.

e. Aspirate and discard the PBS without disturbing the pellet, then resuspend the cells with 0.5 mL of 4°C PBS per 1 × 10^6 cells.

f. Count the cells to ensure the cell density is 1–10,000 cells per 5 µL.

g. Transfer a 5 µL aliquot of each cell suspension to a PCR tube or plate well, then place on ice.

2 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.

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

Component	Volume per sample	Volume for *n* samples[1]
SuperScript™ IV CellsDirect™ Lysis Solution	23.28 µL	*n* × 25.61 µL
Lysis Enhancer (100X)	0.24 µL	*n* × 0.26 µL
DNase I (50X)	0.48 µL	*n* × 0.53 µL
Total volume per sample	**24 µL**	***n* × 26.4 µL**

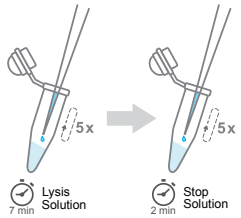
[1] Includes 10% overage.

2 Prepare the Lysis Solution on ice *(continued)*

b. Invert the tube or pipet up and down 5–8 times to mix (do not vortex), then place the Lysis Solution on ice.

c. To prevent loss of DNase I activity, immediately proceed to the next section.

3 Perform cell lysis



a. Add 24 µL of the prepared Lysis Solution to each cell sample-containing tube or well (on ice).

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

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

d. Add 3 µL of SuperScript™ IV CellsDirect™ Stop Solution to each sample.

e. Gently pipet up and down 5–8 times to mix. Do not vortex.

f. Centrifuge briefly to bring the contents to the bottom of the tube or plate well.

g. Incubate for 2 minutes at room temperature, then place the lysates on ice.

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

4 Prepare the reverse transcription reactions

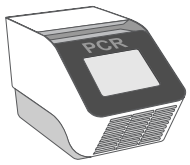
a. Thoroughly vortex the SuperScript™ IV RT Master Mix and SuperScript™ IV No RT Control, then centrifuge briefly to bring the contents to the bottom of each tube.

b. 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.

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

c. Cap the tubes or cover the plates, then immediately perform reverse transcription.

5 Perform reverse transcription



a. Set up the thermal cycling conditions, load the plate or tubes into the thermal cycler, then start the run.

Step	Stage	Temperature	Time
Annealing	1	25℃	10 minutes
Reverse transcription	2	50℃	10 minutes
Enzyme inactivation	3	85℃	5 minutes
Hold	4	4℃	∞

b. At the end of the run, store the cDNA samples as indicated or proceed directly to qPCR or end-point PCR.

Store the single-stranded cDNA on ice for immediate use, at –20℃ for up to 1 week, or at –70℃ 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.

For...	Do this...
qPCR	Prepare the PCR reactions as indicated. • **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. • **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.
End-point PCR	Add the cDNA sample at ≤10% of the PCR reaction volume, or follow the recommendations provided with the reagent used.

Additional reagents

Additional kit reagents can be ordered separately from **www.thermofisher.com**.

Item	Source
SuperScript™ IV CellsDirect™ Lysis Reagent, includes the following: • SuperScript™ IV CellsDirect™ Lysis Solution • SuperScript™ IV CellsDirect™ Stop Solution • Lysis Enhancer	11750550
DNase I	18047019

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**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019141

Revision	Date	Description
A.0	10 March 2020	New document for new product launch.