



Jun 26,
2019

Reverse transcription using SuperScript IV V.2 [↗](#)

Amin Mahpour¹

¹Harvard University

Working [dx.doi.org/10.17504/protocols.io.4stgwen](https://doi.org/10.17504/protocols.io.4stgwen)



Amin Mahpour
Harvard Medical School, Massachusetts General Hospital

EXTERNAL LINK

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205608>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205608>

MATERIALS

NAME	CATALOG #	VENDOR
SuperScript™ IV Reverse Transcriptase	18090050	Thermo Fisher Scientific

- 1 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

Reagent	Amount (uL)
Primer (Random or dT)	0.5
dNTP (10mM)	1
RNA	11

- 2 Incubate the mixture at 72 °C for 00:02:00 . Then, incubate samples on ice for few minutes.



This step allows denaturation of RNA and proper priming for the downstream cDNA synthesis.

- 3 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.



Reagent	Amount (uL)
RT Buffer (5x)	4
DTT(10mM)	1
RNase Inhibitor	1

Add the 6uL to the 12.5uL mix from Step 3.

- 4 Incubate the samples at 37 °C for 00:05:00 . Then, add 1.5uL SuperScript RT IV enzyme to the reaction and mix well.

5 Incubate the samples using the following incubation settings:

Temp (C)	Time (minutes)
25	5
45	40
55	10
75	10

5.1 Add 1uL RNase H to the cDNA samples and incubate at  37 °C for  00:20:00 .

5.2 Dilute the cDNA samples using Nuclease free water.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited