VERSION 2

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Protocol status: Working
We use this protocol and it's working

Reverse transcription using SuperScript IV V.2

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MATERIALS

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SuperScript™ IV Reverse Transcriptase **Thermo Fisher Scientific Catalog**
#18090050

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25139

Keywords: Using SuperScript
VI to produce cDNA from RNA

- 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.

Note

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.