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## cDNA Synthesis Using SuperScript III First-Strand Synthesis System for RT-PCR

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1 Works for me

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### ABSTRACT

The SuperScript® III First-Strand Synthesis System for RT-PCR is used to synthesize first-strand cDNA from purified total RNA. RNA targets from 100 bp to >12 kb can be detected with this system.

The procedure follows the manufacturer's instructions but includes lab specific template and reagent amounts and specifies primers and equipment for use in our lab to prepare total RNA samples for qPCR analysis of gene expression.

The original protocol is attached below:

[superscriptIIIfirststrand\\_pps.pdf](#)

Kit: [Script™ III First-Strand Synthesis System, ThermoFisher Catalog number: 18080051](#)

### DOI

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### EXTERNAL LINK

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### PROTOCOL CITATION

Lynn Doran 2021. cDNA Synthesis Using SuperScript III First-Strand Synthesis System for RT-PCR.

**protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bxa7pihn>

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Invitrogen. "SuperScript III First-Strand Synthesis System for RT-PCR." Jan. 2013. Doc. Part No: 18080051.pps, Pub. No.: MAN0001346, Rev. 3.0. Life Technologies. Lab protocol.

### KEYWORDS

cDNA synthesis, RT-PCR Sample Prep

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### IMAGE ATTRIBUTION

<https://www.thermofisher.com/order/catalog/product/18080051#/18080051>, Invitrogen by Life Technologies.

### CREATED

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### PROTOCOL INTEGER ID

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### GUIDELINES

- Always wear gloves and change them often to avoid RNases.
- Use RNase-free solutions.
- Use RNase-free certified, disposable plasticware and filter tips whenever possible.

## MATERIALS TEXT

- Ice
- Dry Ice
- [Ice Bucket](#)
- [Script™ III First-Strand Synthesis System, ThermoFisher Catalog number: 18080051](#)
- [0.2ml PCR 8-Tube 125 strips flat cap, USA Scientific, 1402-2500](#)
- [Pipette, 1-10 ul, Single Channel, Variable, Eppendorf Research Plus](#)
- [Tips, pipette, 1-10 ul, TIPONE® FILTER TIP REFILLS Item #1121-2710](#)
- [Pipette, 10-100 ul, Single Channel, Variable, Eppendorf Research Plus](#)
- [Tips, pipette, 20- 200 ul, TIPONE® FILTER TIP REFILLS Item #1120-8710](#)
- [RNase Away, spray, ThermoScientific 21-402-178](#)
- [70% Ethanol](#)
- [Thermal cycler, Bio-Rad T100 Thermal Cycler or equivalent](#)
- [Mini-centrifuge, Thermo Scientific™ mySPIN™ 6 Mini Centrifuge](#)

## BEFORE STARTING

RNA extracted from [Qiagen RNeasy Plant Extraction Kit](#), or equivalent quantity and quality.

RNA used for cDNA synthesis for qPCR should be high quality.


- $A_{260}/A_{280}$  values > 1.8, ideally values approach 2.1 (note in SYBR green guide they recommend using samples with >2).
- There is no defined rule for an acceptable  $A_{260}/A_{230}$  ratio for qPCR. A low value is often the result of guanidium thiocyanate contamination from the extraction procedure, and that concentrations of up to [100 mM are tolerated](#). A good rule of thumb,  $A_{260}/A_{230}$  values should be > 1.8, and for pure RNA expect values 2.1-2.3.
- RNA integrity as indicated by Qubit RNA IQ values >5, ideally > 8.

- 1 Allow reagents to thaw completely, mix, and briefly minicentrifuge 10 mM dNTP mix and 50 ng/ul random hexamers before use. Store on ice when not in use.

- 2 Label two PCR tubes per sample, RT and NRT.

Best practice for qPCR is to prepare a no reverse transcriptase (NRT) reaction for each of your samples to ensure that minimal genomic DNA remains in your reverse transcriptase sample and that only cDNA, not gDNA, is being interpreted as gene expression in your qPCR results.


- 3 Treat gloves and pipettes with RNase away and sterilize work area with 70% ethanol before pipetting reagents.

- 4 If performing many reactions, make a master mix of primer random hexamers, dNTP mix, and water, multiply each component by the number of reactions needed plus at least 20% to account for pipetting error. Remember that you will need two reactions for each sample. Pipette  5 µl of the prepared master mix into each sample tube, both RT and NRT.

If only a few reactions are needed, pipette the following for 1 reaction into each tube.



A	B	C
Component	Amount for 1 rxn	Amount for 10 rxn
50 ng/ul Random Hexamers	1 ul	10 ul
10 mM dNTP Mix	1 ul	10 ul
DEPC-treated or MilliQ (18.2 MΩ.cm) water	3 ul	30 ul

- 5 Add  5 µl of high quality RNA from each sample to the respective RT and NRT tubes.


Keep RNA samples on dry ice when not actively in use to inhibit RNases. Return samples to  -80 °C as soon as possible.

- 6 Incubate the tube at  65 °C for  00:05:00 in the thermocycler.

5m

- 7 Place  On ice for a minimum of  00:01:00 .

1m

- 8 Allow reagents to thaw completely, mix, and briefly centrifuge 10X RT buffer, 25 mM MgCl<sub>2</sub>, 0.1 M DTT, 40 U/ul RNase OUT, and 200 U/ul SuperScript III RT before use. Store  **On ice** when not in use.


- 9 Prepare a cDNA Synthesis (RT) Master Mix adding each component in the indicated order. Adjust the table for the number of RT samples.

A	B	C	D
Order	Component	Amount for 1 rxn	Amount for 10 rxn
1	10X RT buffer	2 ul	20 ul
2	25 mM MgCl <sub>2</sub>	4 ul	40 ul
3	0.1 M DTT	2 ul	20 ul
4	40 U/ul RNase OUT	1 ul	10 ul
5	200 U/ul SuperScript III RT	1 ul	10 ul

- 10 Add  **10 µl** of the cDNA Synthesis (RT) Master Mix to each tube labeled RT.

- 11 Prepare a cDNA Synthesis (NRT) Master Mix adding each component in the indicated order. Adjust the table for the number of NRT samples.


A	B	C	D
Order	Component	Amount for 1 rxn	Amount for 10 rxn
1	10X RT buffer	2 ul	20 ul
2	25 mM MgCl <sub>2</sub>	4 ul	40 ul
3	0.1 M DTT	2 ul	20 ul
4	40 U/ul RNase OUT	1 ul	10 ul
5	DEPC-treated or MilliQ (18.2 MQ.cm) water	1 ul	10 ul

- 12 Add  **10 µl** of the cDNA Synthesis (NRT) Master Mix to each tube labeled NRT.



- 13 Program a thermocycler for the following and run all samples, both RT and NRT:


A	B	C
Step	Time (m:s)	Temp (C)
1	10:00	25
2	50:00	50
3	5:00	85
4	Infinity	4

The samples can be removed after the 5 minute incubation at 85°C is complete, they do not need to reach 4°C in the thermocycler.

- 14 Chill  **On ice** until samples are cool to the touch.

- 15 Briefly minicentrifuge all samples.

- 16 Add  **1 µl** of RNase H to each tube (both RT and NRT) and incubate in a thermocycler for  **00:20:00** at  **37 °C**<sup>20m</sup>

- 17 cDNA samples can be stored at  **-20 °C**.