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First Strand cDNA Synthesis Kit using ProtoScript II Reverse Transcriptase (M0368) V.2

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dx.doi.org/10.17504/protocols.io.x14egnmmv5dy/v2**New England Biolabs (NEB)**Tech. support phone: +1(800)632-7799 email: info@neb.comNew England Biolabs
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This protocol explains methods for the "First Strand cDNA Synthesis Kit using ProtoScript II Reverse Transcriptase (M0368)".

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murine RNase inhibitor, transcription, Reverse transcription, RT, cDNA

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MATERIALS

 RNase Inhibitor, Murine - 3,000 units **New England**

Biolabs Catalog #M0314S

 ProtoScript II Reverse Transcriptase - 4,000 units **New England**

Biolabs Catalog #M0368S

 Nuclease-free Water - 25 ml **New England**

Biolabs Catalog #B1500S

 Deoxynucleotide (dNTP) Solution Mix **New England**

Biolabs Catalog #N0447S

- 0.1 M DTT

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 

In a sterile microfuge tube add:

A	B
RNA solution 1ng-1 µg total RNA or 1-100 ng polyA-selected RNA Primer (50 µM dT or 60 µM Random Primer Mix)	2 µl
10 mM dNTP mix	1 µl
Nuclease-free H ₂ O	to final volume of 12 µl

2 

Optional: Heat for 3-5 minutes at  **65 °C** -  **70 °C** . Spin briefly and place promptly  **On ice** .

3 

Add the following (bringing to **20 µl final volume**):

A	B
5X ProtoScript II RT Reaction buffer	4 µl
0.1 M DTT	2 µl
Murine RNase inhibitor (40 U/µl)	1 µl
ProtoScript II Reverse Transcriptase (200 U/µl)	1 µl
<i>Final volume</i>	<i>20 µl</i>

4



If **Random Primer Mix** is used, an incubation step at **25 °C** for **00:05:00** is recommended before the **42 °C** incubation in the next step.

5



Incubate at **42 °C** for 30 minutes-1 hour.

6

Inactivate enzyme at **80 °C** for **00:05:00** .

5m

7



Store products at **-20 °C** or proceed to next step(s).