First Strand cDNA Synthesis (M0368)

New England Biolabs

Abstract

This protocol is for the "First Strand cDNA Synthesis Kit using ProtoScript II Reverse Transcriptase (M0368)".

Citation: New England Biolabs First Strand cDNA Synthesis (M0368). **protocols.io** dx.doi.org/10.17504/protocols.io.chtt6m
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Materials

ProtoScript II Reverse Transcriptase - 4,000 units M0368S by New England Biolabs

Protocol

Step 1.
In a sterile microfuge tube add:

PROTOCOL

. M0368 RNA and Primer mixture

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ANNOTATIONS
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Optionally, heat for 3-5 minutes at 65-70°C. Spin briefly and place promptly on ice.

Step 1.1.
RNA solution 1ng-1 µg total RNA or 1-100 ng polyA-selected RNA

Step 1.2.
Primer (50uM dT or 60uM Random Primer Mix) **2 µL**

Step 1.3.
10mM dNTP mix **1 µL**

Step 1.4.
Nuclease-free H2O to **final volume of 12 µL**

Step 2.
Add the following (bringing to **20 µL final volume**):

PROTOCOL

. **M0368 RT Mixture**

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Step 2.1.

5X ProtoScript II RT Reaction bufferr **4 µL**

Step 2.2.

0.1M DTT **2ul**

Step 2.3.

murine RNAse inhibitor (40U/ul) **1 µL**

Step 3.

Incubate at 42°C for 30min-1hr.

DURATION

01:00:00

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If Random Primer Mix is used, an incubation step at 25°C for 5 minutes is recommended before the 42°C incubation.

Step 4.

Inactivate enzyme at 80°C for 5 minutes.

DURATION

00:05:00

Step 5.

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