First Strand Synthesis with Reverse Transcriptase

New England Biolabs

Abstract

This protocol is for First Strand Synthesis with Reverse Transcriptase

Citation: New England Biolabs First Strand Synthesis with Reverse Transcriptase. **protocols.io** dx.doi.org/10.17504/protocols.io.cjuunv
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Protocol

Step 1.
In a sterile microfuge tube add the following:

PROTOCOL

. FirstStrand with RT Reaction

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Step 1.1.
RNA solution 0.5-2 µg (total RNA or 50-100 ng polyA-selected RNA)

Step 1.2.
Primer (dT or N9) **2 µL**

Step 1.3.
dNTP mix **4 µL**

Step 1.4.
nuclease-free H2O to final volume of **16 µL**

Step 2.
Heat for 3-5 minutes at 65-80°C.

DURATION
00:05:00

Step 3.
Spin briefly and place promptly on ice.

Step 4.
Add 10X RT Buffer

AMOUNT
2 µl Additional info:

PROTOCOL

. 10X RT Buffer

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Step 4.1.
500 mM Tris-HCl (pH 8.3 @ 25°C)

Step 4.2.
750 mM KCl

Step 4.3.
30 mM MgCl2

Step 4.4.
100 mM DTT

Step 5.
Add RNAse inhibitor **1 µL**

Step 6.
Add M-MuLV Reverse Transcriptase **1 µL**

Step 7.
Incubate at 42°C for one hour.

DURATION
01:00:00

Step 8.
Inactivate enzyme at 90°C for 10 minutes.

DURATION
00:10:00

Step 9.
Store products at -20°C or proceed to next step(s).