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Feb 15, 2022

First Strand Synthesis with Reverse Transcriptase (M0253) V.2

New England Biolabs[1]

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This protocol is for First Strand Synthesis with M-MuLV Reverse Transcriptase (M0253).

DOI

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M-MuLV reverse transcriptase, first strand reverse transcription, transcription

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MATERIALS

Magnesium Chloride **Fisher Scientific Catalog #AC223210010**

DTT (Dithiothreitol) (> 99% pure) Protease free **Gold Biotechnology Catalog #DTT**

Tris-HCl **Life Technologies Catalog #AM9855**

Potassium chloride **Sigma Aldrich Catalog #P9333**

M-MuLV Reverse Transcriptase **New England Biolabs Catalog #M0253**

Materials needed:

10X RT buffer:

A	B
Tris-HCl (pH 8.3 @ 25°C)	500 mM
KCl	750 mM
MgCl2	30 mM
DTT	100 mM

dNTP mix (2.5 mM each in water titrated by Tris-HCl to pH 7.0)

Oligo-dT primer (40 µM)

Random nonamers (40 µM)

RNase Inhibitor (10 U/µL)

M-MuLV Reverse Transcriptase (200 units/µL)

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

Prepare the following solutions:

10X RT buffer:

A	B
Tris-HCl (pH 8.3 @ 25°C)	500 mM
KCl	750 mM
MgCl2	30 mM
DTT	100 mM

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dNTP mix (2.5 mM each in water titrated by Tris-HCl to pH 7.0)

1 In a sterile microfuge tube add the following:

Reagent	Volume
RNA solution	0.5-2 µg (total RNA) or 50-100 ng (PolyA-selected RNA)
Primer (Oligo-dT primer at 40 µM or Random nonamer N9 at 40 µM)	2 µL
dNTP mix (2.5 mM each in water titrated by Tris-HCl to pH 7.0)	4 µL
nuclease-free H2O	to final volume of 16 µL

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

3 Spin briefly and place promptly **On ice** .

4 

Add:

2 µL 10X RT buffer

1 µL RNAse inhibitor

1 µL M-MuLV Reverse Transcriptase

Final volume: **20 µL**

5 

Incubate at **42 °C** for **01:00:00** .

6 Inactivate enzyme at **90 °C** for **00:10:00** .

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

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