M-MuLV Reverse Transcriptase

Ordering Info

TBZ0321, 20,000 U (200 U/ µL)

TBZ0322, 100,000 U (200 U/ µL)

Description

M-MuLV Reverse Transcriptase is a recombinant reverse transcriptase from Moloney Murine Leukemia Virus produced in *Escherichia coli*. Highly purified the enzyme is an useful RNA-dependent DNA polymerase to synthesize cDNA using a RNA template and an oligo(dT) primer or a specific reverse primer.

CAS: 9068-38-6

MW: 69 kDa

Features

- The enzyme lacks of 3´→5´ and 5´→3´ exonuclease activity.
- The enzyme has not RNase H activity.

Applications

- First-strand cDNA synthesis experiments
- RT-PCR
- RT-qPCR

Quality Control

Functionally tested in a quantitative RT-PCR of actin gene using as template dilutions of total RNA transcribed using 200 units of enzyme.

Kit Components

Components	TBz0321	TBZ0322
M-MuLV Reverse Transcriptase (200 U/ µL)	100 µL	500 µL
M-MuLV Reaction Buffer (10x)	1.5 mL	2 x 1.5 mL

Order Info Kit Components: *M-MuLV Reaction Buffer (10x) (TBB0317).*

Storage

Store at -20°C. Shipped in blue ice.

Unit Definition

One unit is defined as the amount of enzyme required to incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37 °C using poly(A) : oligo (dT) as template : primer.

Material required (not supplied)

- dNTPs mix 2.5 mM each (TBR0209)
- PCR Grade Water (TBB0303)
- Ribonuclease Inhibitor
- PCR Tubes
- Oligo(dT) and specific primers

PROTOCOL

1. Thawing all components on ice. Vortex them and centrifugate.

2. On ice, prepare a mix of the following components,

Reaction Components	Final Concentration	Volume
RNA template	*Total RNA*: 10 pg - 5 µg *mRNA*: 10 pg - 0.5 µg	x µL
dNTPs Mix 40 mM TOTAL	0.5 mM	1 µL
Oligo(dT)$_{12-18}$ (50-60 µM) *or* Specific Reverse Primer (2 µM) *or* Random Hexamer (50-250 ng/ µL)	2.5 – 3 µM 0.1 µM 2.5 – 12.5 ng/ µL	1 µL
Nuclease free Water		up 16 µL
Final Volume		**16 µL**

3. [Optional] Incubate at 65 °C, 5 minutes.

   This step is recommended for sequences with high GC content.

4. Spin the tubes and add the following components on ice:

Reaction Components	Final Concentration	Volume
M-MuLV Reaction Buffer (10x)	1 x	2 µL
Ribonuclease Inhibitor		1 µL
M-MuLV Reverse Transcriptase (200 U/ µL)	10 U/ µL	1 µL
Final Volume		**20 µL**

5. Incubate at 37 °C, 50 minutes.

   If you are using random hexamers, firstly incubate at 25°C, 15 minutes and then at 37 °C, 50 minutes.

6. To inactivate the enzyme, incubate at 70°C, 15 minutes.

7. Store the synthetized cDNA at -20°C.