SinaClon
شرکت سیناکلون BioScience

M-MuLV Reverse Transcriptase

(10000 units)

REF **MO5431** **Concentration: 200u/µl**

Wet or Dry ice **Store at: -20°C**

RUO

Components

Contents	Quantity/ Volume	Store Temperature
Reverse Transcriptase (200u/µl)	50µl	-20°C
5X Reaction Buffer	400µl	-20°C

Description

M-MuLV Reverse Transcriptase is a recombinant Enzyme with reduced RNase H activity and increased thermostability. The enzyme is active up to 55°C. It provides higher specificity, higher yield and more full-length cDNA products.

Features

- Increased thermostability for more full-length cDNA products.
- Deficient RNase H activity to reduce RNA template degradation during the first-strand cDNA synthesis.
- cDNA up to 15 kb.

Unit Definition

One activity unit (U) refers to the amount of M-MuLV reverse transcriptase when catalyzes the incorporation of 1nmol of dTTP into materials in 10min at 37°C using oligo (dT) primed poly (A) as a template.

Reaction Buffer

250mM KCl; 15mM $MgCl_2$; 10mM DTT; 100mM Tris-HCl pH 8.4.

First Strand cDNA synthesis (20µl reaction volume)

1. Add components according to the below table:

Components	Volume
Total RNA/mRNA	50ng-5µg / 5-500ng
Oligo(dT)18 (0.5µg/µl) Or Random Primer (0.1µg/µl) Or GSP (Gene Specific Primer)	1µl 1µl 2pmol
dNTP Mix, 10 mM each	1µl
5 X RT Buffer	4µl
Ribonuclease Inhibitor (40 units/µl)	0.5µl
M-MuLV Reverse Transcriptase	1µl
RNase free H_2O to final volume	20µl

Optional (if RNA template is GC-rich or is known to contain secondary structures).

Suggest to mix RNA/ Primer/ RNase free H_2O gently and briefly centrifuge, incubate at 65°C for 5min, chill on ice and briefly centrifuge, then place the tube on ice. Add other components and continue.

2. Mix well gently

If Oligo(dT)$_{18}$ or gene specific primer (GSP) are used, incubate at 50°C for 30-50min.
If Random Primer is used, incubate 10min at 25°C followed by 30-50min at 50°C.

3. Terminate the reaction by heating at 70°C for 15min.

The reverse transcription reaction product can be directly used in PCR or stored at -20°C.

RT-PCR
Use 2-4µl of the reaction mix to perform PCR in 50µl volume.

PCR mixture set up (for 50µl reaction volume)

Components	Volume	Final Concentration
cDNA Template	2-4µl	as required
Forward Primer (10µM)	1µl	0.2µM each
Reverse Primer (10µM)	1µl	0.2µM each
10X Taq Buffer (contains Mg^{2+})	5µl	1X
2.5mM dNTPs	4µl	0.2mM
Taq DNA Polymerase	0.5µl	2.5 units
ddH2O to final volume	50µl	Not applicable

PCR Condition

94°C	2-5 min	1 Cycle
94°C	30 sec	30-40 Cycles
Variable	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	1 Cycle

Quality Control
Purified free of detectable levels of RNase, endonuclease and exonuclease activities.

Signs

Signs	Definitions	Signs	Definitions
RUO	For Research Use Only		Name and address of the manufacturer of the product
	Product shipping conditions	REF	Product technical code

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