Tehran Cavosh Clon
Our Aim is Your Success

M-MuLV Reverse Transcriptase (10000 units)

For Research Use Only

Cat. No.: MO5431 **Store at -20°C**
Concentration: 200u/µl
Supplied with: 400µl of 5X Reaction Buffer

Description

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

- 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-MLV reverse transcriptase when catalyzes the incorporation of 1 nmol of dTTP into materials in 10 min at 37℃ using oligo (dT) primed poly (A) as a template.

5 X RT Buffer

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

First Strand cDNA synthesis (20 µl reaction volume)

1. Add components according to the below table:

Components	Volume
Total RNA/mRNA	50 ng-5 µg/5-500 ng
Oligo$(dT)_{18}$ (0.5 µg /µl)	1 µl
Or random Primer (0.1 µg/µl)	1 µl
Or GSP (Gene Specific Primer)	2 pmol

dNTP Mix, 10 mM each	1 µl
5 X RT Buffer	4 µl
Ribonuclease Inhibitor (40 units/µl)	0.5 µl
M-MuLV Reverse Transcriptase	1 µl
RNase free H_2O to final volume	20 µl

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℃ for 5 min, 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℃ for 30-50 min.

If Random Primer is used, incubate 10 min at 25℃ followed by 30-50 min at 50℃.

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

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

RT-PCR

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

PCR mixture set up (for 50 µl reaction volume)

Components	Volume	Final Concentration
cDNA Template	2-4 µl	as required
Forward Primer (10 µM)	1 µl	0.2 µM each
Reverse Primer (10 µM)	1 µl	0.2 µM each
10X Taq Buffer (contains Mg^{2+})	5 µl	1×
2.5 mM dNTPs	4 µl	0.2 mM
Taq DNA Polymerase	0.5 µl	2.5 units
ddH_2O to final volume	50 µl	Not applicable

PCR Condition

94℃	2-5 min	
94℃	30 sec	30-40 cycles
50-60℃	30 sec	
72℃	1-2 kb/min	
72℃	5-10 min	

Quality Control

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

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