M-MuLV Reverse Transcriptase (10000 units)

Cat. No.: MO5431 **Store at -20°C**
Concentration: 200u/µl
Supplied with: 500µl of 10X Reaction Buffer

Description
Molony Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (RT) is an RNA-dependent DNA polymerase. It can synthesize a complementary DNA strand initiating from a primer using either RNA or single-stranded DNA as a template. The absorbance of RNase H activity enhances the synthesis of long cDNA and therefore the enzyme is recommended for preparing long cDNA.

Definition of Activity Unit
One unit of the enzyme incorporates 1nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA), $Oligo(dT)_{18}$

10X Reaction Buffer
50mM Tris-HCl (pH 8.3 at 25°C), 75mM KCl, 3mM $MgCl_2$ and 10mM DTT

Assay Condition
50mM Tris-HCl (pH 8.3), 6mM $MgCl_2$, 10mM DTT, 0.4mM poly(rA) , $Oligo(dT)_{12-18}$ and 0.5mM [^{3}H] dTTP in a reaction volume of 50µl.

Applications
- First strand cDNA synthesis for RT-PCR and real-time RT-PCR.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- DNA labeling.
- Analysis of RNA by primer extension.

Storage Buffer
10mM K-Phosphate(PH 7.5), 0.1mM EDTA, 200mM NaCl, 7mM 2-mercaptoethanol and 50% (v/v) glycerol

Protocol for First Strand cDNA Synthesis
The following protocol is optimized to generate first-strand cDNA for use in two-step RT-PCR.
Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add into sterile, nuclease-free tube on ice in the indicated order:

	total RNA	0.1ng-5µg
Template RNA	poly(A) RNA	10pg-500ng
	specific RNA	0.01pg-0.5 µg
	$Oligo(dT)_{18}$	0.5µg (100 pmol)
Primer	Random hexamer	0.2µg (100 pmol)
	Gene specific primer	15-20 pmol
DEPC-treated water		to 12.5 µl

2. **Optional**: If RNA template is GC rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min, chill on ice, briefly centrifuge and place on ice.

3. Add the following components in the indicated order:

10X Reaction Buffer	2µl
RiboLockRNase Inhibitor	0.5µl (20u)
dNTP Mix, 10mM each	2µl (1mM final concentration)
RevertAid Reverse Transcriptase	1µl (200u)
Total volume	20µl

Mix gently and centrifuge briefly.

4. If oligo$(dT)_{18}$ primer or gene-specific primer is used, incubate 60 min at 42°C.
If random hexamer primer is used, incubate 10 min at 25°C followed by 60 min at 42°C.
For transcription of GC rich RNA reaction temperature can be increased to 45°C.

5. Terminate the reaction by heating at 70°C for 10 min. Do not heat-inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

Note
• The reverse transcription reaction product can be directly used in PCR or stored at -20°C.
• Use 2 µl of the reaction mix to perform PCR in 50 µl Volume.

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

For Research Use Only

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