

MBT072

M-MuLV Reverse Transcriptase

Product Name	Product Code	Kit Packing
M-MuLV Reverse Transcriptase	MBT072-1000U	1000 units
	MBT072-5000U	5000 units

Description:

M-MuLV Reverse Transcriptase (Molony Murine Leukemia Virus Reverse Transcriptase) is an RNA-dependent DNA polymerase requiring a DNA primer and an RNA template to synthesize a complementary DNA strand. M-MuLV Reverse Transcriptase has a weaker intrinsic RNase H activity than AMV RTase (Avian Myeloblastosis Virus Reverse Transcriptase). The absence of RNaseH activity enhances the synthesis of long cDNAs and therefore the enzyme is recommended for preparing long cDNAs.

Applications:

1. First strand cDNA synthesis
2. DNA Labelling
3. RNA analysis by primer extension

Unit Definition:

1U is defined as amount of enzyme that is required to catalyze the incorporation of 1 nmole of dNTP into acid-insoluble material in 10 minutes at 37°C using poly (A)-oligo (dT) as template-primer.

Concentration: 200 units/μl supplied with reaction buffer

Thermal Inactivation: 70°C for 10 minutes

Storage conditions: The M-MuLV Reverse Transcriptase should be stored at -20°C. When stored under the recommended conditions, the product is stable for 18 months.

General Reaction Protocol:

1. Mix the template RNA and the primer in RNase-free tube.
NOTE: Concentration of template RNA and primer (20μl reaction volume)

Template RNA	Total RNA	10 ng-5 μg
	Poly(A) ⁺ mRNA	5 ng-0.5 μg
Primer	Oligo(dT)	0.5 μg
	Random hexamer	0.2 μg
	Sequence specific Primer	15-20 pmole
RNase-free water (DEPC-treated water)	-	Upto 10 μl

Please refer disclaimer Overleaf.



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2. Incubate the mixture at 70°C for 5 minutes and chill on ice.
3. Add 4 µl of reaction buffer, 2µl of 10mM dNTP mixture and 20 units of RNase inhibitor and RNase free (DEPC-treated) water upto 19 µl.
4. Incubate at 37°C for 5 minutes. If random primers are used, incubate at 25°C for 5 minutes.
5. Add 1 µl (200 units) of M-MuLV Reverse Transcriptase.
6. Mix by gently pipetting up and down (total reaction volume 20µl)
7. Incubate at 37°C-42°C for 60 minutes.
8. Stop the reaction by heating at 70°C for 10 minutes. Chill on ice.
NOTE: To perform PCR, add the finished RT reaction upto 1/5th of final PCR volume.

Quality control:

Detected free of RNases, endonuclease and exonuclease activities.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

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