

MBT072

**M-MuLV Reverse Transcriptase (RNase H-)
(Concentration: 200 units/μl)**

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.

Kit contents

Components	Product Code	Reagents provided for (reactions)	
		1000U	5000U
M-MuLV Reverse Transcriptase (RNase H-)	MBT072	5 μL	25 μL
RT Buffer for MMuLV	DS0279	30 μL	130 μL
10X Solution	DS0280	15 μL	65 μL

Materials needed but not provided for cDNA synthesis:

- Random Hexamer,
- Oligo (dT)
- Ribonuclease Inhibitor
- 10 mM dNTP Mix (Product code: MBT078)
- Molecular Biology Grade Water for PCR (Product code: ML065)
- Thermal cycler
- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

Applications:

1. First strand cDNA synthesis
2. Preparation of cDNA Probe
3. For RT-PCR & Real time RT-PCR

Unit Definition:

1U is defined as amount of enzyme that is required to catalyze the incorporation of 1 nmoles 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

Storage conditions: The M-MuLV Reverse Transcriptase should be stored at -20°C. When stored under the recommended conditions, the product is stable for 12 months.

Procedure for cDNA synthesis:

1. Add the reagents as follows:

Ingredients	Volume per reaction (Any of the below combinations can be followed)		
	Random Hexamer	Oligo(dT)	Random Hexamer:Oligo(dT) Mix
Random Hexamer*	1 µL	-	-
Oligo(dT)*	-	1 µL	-
Random Hexamer : Oligo(dT) Mix*	-	-	2 µL
RNA template	5 ng to 5 µg		
Molecular Biology Grade Water for PCR	Up to 10 µL		

*Alternatively, one can use a gene-specific reverse transcription primer.

2. Incubate for 5 min at 65°C, then cool immediately on Ice.

3. Prepare the reaction mixture in a total volume of 20 µL.

Components	Code	Volume per reaction
Template RNA Primer Mixture (from step 2)	-	10 µL
RT Buffer for M-MuLV	DS0279	4 µL
10X Solution for M-MuLV	DS0280	2 µL
M-MuLV Reverse Transcriptase (RNase H-)	MBT072	1 µL
Ribonuclease Inhibitor	-	0.5 µL
10 mM dNTP mix	-	2 µL
Molecular Biology Grade Water for PCR	-	Up to 20 µL

4. Gently mix and ensure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

5. For preparation of cDNA using, incubate the complete reaction mix as follows:

- a. For preparation of cDNA using, incubate the complete reaction mix using Random Hexamer

Random Hexamer	No. of cycles
25°C for 5 minutes	1 cycle
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

OR

- b. For preparation of cDNA using, incubate the complete reaction mix using For Oligo (dT)

Oligo(dT)	No. of cycles
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

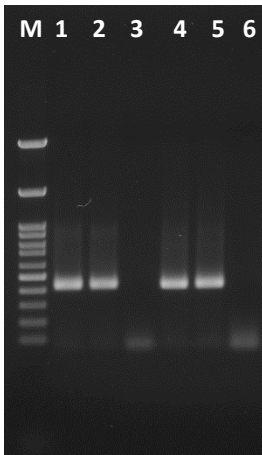
OR

c. For preparation of cDNA using, incubate the complete reaction mix using Random Hexamer : Oligo(dT) Mix

Random Hexamer:Oligo(dT) Mix	No. of cycles
25°C for 5 minutes	1 cycle
42°C for 60 minutes	1 cycle
70°C for 5 minutes	1 cycle
Hold at 4°C	optional

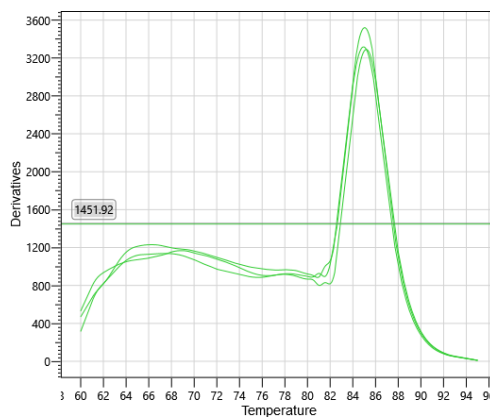
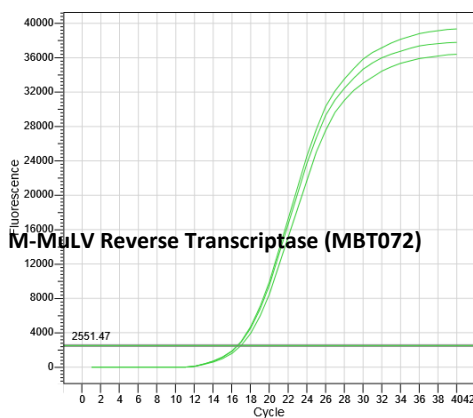
6. The cDNA can be further used to perform conventional or Real-Time PCR assay.

Amplification Data



Lane	Sample
M	100bp DNA ladder
1,2	Amplicon obtained using Random Hexamer
4,5	Amplicon obtained using Oligo(dT)
3,6	Negative controls

Representative data of Semi-quantitative PCR of cDNA synthesized using after amplification



Ct value	Tm
17.01	85.2
16.64	85
16.7	84.9

Representative data of Real-time SYBR based PCR of cDNA synthesized using M-MuLV Reverse Transcriptase (MBT072) after amplification

Quality control

Detected free of RNases, endonuclease and exonuclease activities.

Warning

Not for Medicinal Use

Precautions

Read the procedure carefully before starting the experiment. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Troubleshooting Guide:

Sr. No.	Problem	Possible cause	Possible solution
1	No amplification product	No cDNA synthesis (temperature too high)	For the cDNA synthesis step, incubate <50°C.
		RNase contamination	Maintain aseptic conditions; add Ribonuclease inhibitor
		Not enough starting template RNA	Increase the concentration of template RNA
		RNA has been damaged or degraded	Replace RNA if necessary
		RT inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Note: Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine
		Annealing temperature is too high	Decrease temperature as necessary
		Extension time is too short	Set extension time for at least 60 seconds per kb of target length
		Cycle number is too low	Increase cycle number
2	Low specificity	Reaction conditions not optimal	<ul style="list-style-type: none">• Optimize magnesium concentration• Optimize the primer• Optimize the annealing temperature and extension time• Increase temperature of RT reaction to 60°C
		Oligo(dT) or Random primers used for first-strand synthesis	Use only gene-specific primers
3	Unexpected bands after electrophoretic analysis	Contamination by genomic DNA	<ul style="list-style-type: none">• Pretreat RNA with DNase I
		Nonspecific annealing of primers	<ul style="list-style-type: none">• Vary the annealing temperature• Optimize the magnesium concentration for each template
		Primers formed dimers	Design primers without complementary sequences at the 3' ends




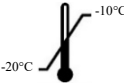




Safety Information

The M-MuLV Reverse Transcriptase (RNase H-) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

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.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBT072

Rev.No.:09

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Disclaimer :

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