For research use only

ISO9001

M-MLV Reverse Transcriptase, Thermostable

Product	Quantity	Cat. No.	Remarks
M-MLV Reverse Transcriptase	10,000 U	EBT-1503	200 U/μl, RNase H$^-$
Thermostable	50,000 U	EBT-1504	200 U/μl, RNase H$^-$

Description:

M-MLV (Moloney Murine Leukemia Virus) Reverse Transcriptase is an RNA-dependent DNA polymerase that can be used in first strand cDNA synthesis using total or poly A$^+$ RNA as templates. M-MLV Reverse Transcriptase is purified from a recombinant *E.coli* clone that carrying *pol* gene of M-MLV and consists of a single subunit with a molecular weight of 71 kDa.

Thermostable M-MLV Reverse Transcriptase RNase H minus product is a genetically modified enzyme to remove the associated RNase H activity and give thermal stability. It can be used in cDNA synthesis with long messenger RNA templates (>5 kbp).

Storage Buffer:

200 unit/μl in 20 mM Tris-HCl, pH7.5, 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP-40, and 50% Glycerol.

Application(s):

First strand cDNA synthesis from total RNA or polyA$^+$ RNA, Primer extension, RT-PCR.

Unit Definition:

One unit is the amount of enzyme required for incorporation of 1 nmole of dNTP into acid-insoluble material for 10 min at 37℃.

5x Reaction Buffer:

250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT.

QC tests:

Activity, SDS-PAGE purity, endonuclease activity test, performance tests.

Storage Condition::

Store at -20℃.

Heat Inactivation:

M-MLV reverse transcriptase can be inactivated by incubation at 90°C for 10 min.

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First Strand cDNA Synthesis:

1. Add 1 μl of 100 pmol/μl primer (random hexamer or oligo d$(T)_{15}$) or 10 pmol/μl of gene specific anti-sense primer per microgram of the total RNA sample in a total volume of ≤10 μl in nuclease-free water.
2. Heat the tube to 70°C for 5 min to melt secondary structure within the RNA template.
3. Cool the tube immediately on ice, then spin briefly to collect the solution at the bottom of the tube.
4. Add the followings to the primer/RNA mix in the order shown.

M-MLV 5X Reaction Buffer	4 μl
dNTP mix (2.5 mM each)	4 μl
M-MLV RT 200 units	1 μl
Add nuclease-free DW to final volume of 20 μl	

5. Mix gently by flicking the tube, and incubate for 60 min at 50°C or 55°C.

(considering low bindng efficincy of random or oligo dT primers at high temperature, specific anti-sense primer in RT reaction may be helpful)

6. To stop reaction, incubate for 5 min at 94°C.

Notes:

- The cDNA by reverse transcription can be used for subsequent PCR reactions and for the cDNA library constructions.
- If there is concern about possible RNase contamination in the reaction, Recombinant RNase Inhibitor may be added to the reaction to preserve RNA integrity.

PCR:

1. Add the following components to the PCR tubes (for 20 μl total reaction).

10x PCR Buffer	2 μl
dNTP mix (2.5 mM each)	1.6 μl
Primers (10 pmol/μl)	0.5 μl each
cDNA by RT reaction	0.1-1 μl
Taq (5 unit/μl)	0.2 μl
Add nuclease-free DW to final volume of 20 μl	

2. Perform PCR reaction as follows.

PCR conditions		(100bp – 1kb)		(1-3kb)	
	94℃	5 min		5 min	
	94℃	30 sec	25-40	30 sec	25-40
	50-60℃[a]	30 sec		30 sec	
	72℃	45 sec		1.5 min	
	72℃[b]	5 min		5 min	

a, Optimal annealing temperature is dependent on the melting point of primer pair
b, Final extension at 72°C can be omitted if the purpose of PCR is not for a TA cloning
c, The number of PCR cycle is dependent on a copy number of target mRNA. For a rare copy gene, increase cycle number.