M-MuLV Reverse Transcriptase

Cat. No.: E00050 Version 2018-07-16

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I Description

GenScript M-MuLV Reverse Transcriptase (M-MLV) is derived from a cloned region of the *pol* gene of MMLV and isolated from an *E. coli* strain overexpressing this construct. To increase cDNA yields and get a higher percentage of longer transcripts, the M-MLV Reverse Transcriptase has been modified with reduced RNase H activity, and expressed free of exogenous RNases and other nucleases. The enzyme can synthesize a complementary cDNA strand initiating from a primer using RNA as template (cDNA synthesis), making it ideal for a wide range of applications.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the transfer of 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C.

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II Kit Contents

Kit Content	Unit Concentration	Amount	
		50-reaction	200-reaction
M-MuLV Reverse Transcriptase	200 U/μL	10000 U	40000 U
5X Reaction Buffer	250 mM Tris-HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM $MgCl_2$	1.0 mL	1.0 mL
DTT	100 mM	0.25 mL	1 mL

***Materials required but not provided**

1. RNase inhibitor
2. dNTP mixture
3. Oligo(dT)20 , random 6-mers or gene-specific reverse primer
4. RNase free dH_2O
5. PCR thermal cycler, agarose gel, microcentrifuge, electrophoresis apparatus, micropipettes and pipette tips (nuclease free)

III Certificate ofAanalysis

◇ **High Protein Purity:** M-MLV is > 95% pure as determined by SDS-PAGE with Coomassie Blue detection.

◇ **Non-specific Endonuclease Activity:** A 20 μL reaction in M-MLV reaction buffer containing 200 ng of supercoiled ΦX174 RF DNA and 200 U of M-MLV, incubated for 2 h at 37°C. < 20% conversion of ΦX174RF DNA to Form II and no conversion to Form III.

◇ **Non-specific Exonuclease Activity:** A 20 μL reaction in M-MLV reaction buffer containing 200 ng of 500 bp labeled double-stranded DNA and 200 U of M-MLV, incubated for 16 h at 37°C. No DNA degradation is determined by agarose gel electrophoresis.

◇ **Non-specific RNase Activity:** A 20 μL reaction in M-MLV reaction buffer containing 2000 ng of total RNA and 200 U of M-MLV incubated for 2 h at 37°C. No RNA degradation as determined by agarose gel electrophoresis.

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IV Storage

M-MuLV Reverse Transcriptase is supplied with 1x storage buffer (20 mM Tris-HCl, 100 mM NaCl, 0.01% NP-40, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol pH 7.5 at 25°C). The recommended storage temperature is -20°C. Guaranteed stable for 12 months when stored properly.

V Protocol

Reverse Transcription of RNA (First Strand Synthesis)

Prepare the following mixture in a 0.2 mL nuclease-free tube.

Component	Volume
50 μM oligo(dT)20 primer, 50 μM Random 6mers , or 2 μM gene-specific reverse primer	1 μL (2 μL for Random 6-mers)
10 mM dNTP mix (10 mM each)	1 μL
Template RNA	10 pg–5 μg total RNA or 10 pg–500 ng mRNA
RNase Free dH2O	to 12 μL

Mix, spin briefly and heat for 5 min at 65-70°C, and then incubate immediately on ice.

Add remaining components (final volume 20 μL).

Component	Volume
Template RNA Primer Mixture (from step 2)	12 μL
5X Reaction Buffer	4 μL
DTT	2 μL
RNase Inhibitor	1 μL
M-MuLV Reverse Transcriptase	1 μL

Incubate reactions:

1. If using oligo(dT)20 or gene-specific primer, directly proceed to step 3.

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2. If using random 6-mers, incubate the combined reaction mixture at 23°C for 10 min, and then proceed to step 3.
3. Mix gently, spin briefly, and incubate at 42°C for 60 min.

Note: In general, reactions should be performed at 42°C. However, for RT-PCR reactions where the genespecific primer is used for cDNA synthesis, we recommend performing the reverse transcription reaction at 50°C to reduce the possibility of non-specific amplification products.

Inactivate reactions:

1. Incubate at 92°C for 10 min to inactivate the M-MLV Reverse Transcriptase, then cool on ice.
2. For synthesis of longer cDNAs (> 4 kb), inactivation at 70°C for 15 min is recommended to minimize cDNA damage.

VI Example

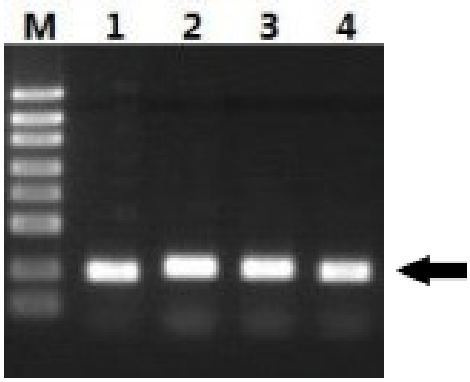

Figure 1: **Competitive performance of M-MLV Reverse Transcriptase.** A 0.5 kb RT−PCR product is obtained from 100 ng of total mouse liver RNA using β−actin primers. Compared with competitors, GenScript M-MLV displays efficient amplification performance. M: Marker; lane 1: 200 U M-MLV from competitor; lane 2: 500 U GenScript M-MLV; lane 3: 200 U GenScript M-MLV; lane 4: 100 U GenScript M-MLV.

VII References

1. Verma, I.M. (1975). J. Virol. 15, 843-854.
2. Gerard, G.F. and Grandgenett, D.P. (1975). J. Virol. 15, 785-797.
3. Roth, M.J., Tanese, N. and Goff, S.P. (1985). J. Biol. Chem. 260, 9326-9335.

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VIII Ordering information

Product Name	Cat. No.
dNTP (10 mM each) ≥99% HPLC	C01582
dNTP mixture, 10 mM each	D0056

* Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans.

Contact us

Web: https://www.genscript.com
Email: product@genscript.com
Fax: 1-732-518-5150

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