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MMLV Reverse Transcriptase

Cat. Nos. M6125H, M6110K, M4425H, and M4410K

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1. Introduction

MMLV Reverse Transcriptase, encoded by Moloney Murine Leukemia Virus (MMLV RT) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template to which a primer has been hybridized.[1-3] MMLV RT will also extend primers hybridized to single-stranded DNA. Second strand cDNA synthesis can be achieved from some mRNA templates without an additional DNA polymerase. As little as 5 units of our MMLV RT per µg of RNA template synthesizes full-length cDNA at levels comparable to that of modified (RNase H^-) MMLV RTs.

2. Applications

Synthesis of cDNA.[4-6] The most common use of MMLV RT is for first-strand cDNA synthesis. This is the first step in the cloning of specific cDNAs primed with oligonucleotides specific to the mRNA of interest, and in the construction of cDNA libraries from poly(A)$^+$ RNA primed with oligo(dT). Although MMLV RT can also synthesize the second strand, most protocols employ another enzyme such as *E. coli* DNA polymerase I for second-strand synthesis.

mRNA 5′-end Mapping by Primer Extension Analysis.[4] The 5′end(s) of specific mRNA molecules can be mapped and quantitated by using MMLV RT to extend a ^{32}P-5′-end-labeled primer hybridized to the mRNA. By examining the resulting cDNA by autoradiography of a polyacrylamide gel, the researcher can determine the number, location and relative abundance of transcriptional start sites.

RT-PCR.[4] The cDNA synthesized by MMLV RT from RNA of a known sequence can be used as a template in the Polymerase Chain Reaction* for geometric amplification of a target sequence.

Dideoxynucleotide Sequencing.[4,7] MMLV RT is occasionally used to sequence homopolymeric A/T or G/C regions which may be difficult to sequence with other DNA polymerases, particularly the Klenow fragment of *E. coli* DNA Polymerase I.

End-labeling of DNA.[4] MMLV RT can be used to radioactively label DNA fragments with a 5′-protruding termini in a fill-in reaction using the appropriate radioactive dNTP(s).

Synthesis of Radioactive cDNA Probes.[4] Uniformly-labeled DNA probes can be made from RNA templates by hybridizing either specific or random oligonucleotide primers to the RNA and extending the primers with MMLV RT in the presence of a radioactively labeled dNTP.

Successful cDNA synthesis requires consideration of the character and activity of both endogenous and contaminating RNase activities. Unmodified MMLV RT has a weak endogenous RNase H activity that degrades the RNA strand of an RNA:DNA hybrid. This RNase H activity is much weaker than that of the reverse transcriptase derived from Avian Myeloblastosis Virus (AMV). The high level of RNase H associated with AMV RT limits the length and total yield of cDNA that can be made with the avian enzyme, making MMLV RT the preferred enzyme for most applications. MMLV RT does not have endogenous DNA exonuclease activity. Our preparation of MMLV RT is free of exogenous RNase, a common contaminant in commercial preparations of reverse transcriptase.

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: MMLV RT is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol (DTT), 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of MMLV RT catalyzes the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using oligo$(dT)_{12\text{-}18}$-primed poly$(A)_n$ as a template.

Note: The unit assay conditions are considerably different from the recommended reaction conditions described below.

Activity Assay: The unit definition assay is performed in a reaction containing 50 mM Tris-HCl (pH 8.6), 40 mM KCl, 1 mM $MnSO_4$, 1 mM DTT, 200 mM poly(A):oligo-$(dT)_{12\text{-}18}$ (1:1 molar ratio), and 0.5 mM dTTP.

10X Reaction Buffer: 0.5 M Tris-HCl (pH 8.3), 0.1 M $MgCl_2$, and 0.75 M KCl.

Contaminating Activity Assays: MMLV RT is free of detectable RNase and DNase (exo- and endonuclease) activities.

4. Related Products

The following products are also available:

- dNTP Solutions
- MasterAmp™ RT-PCR Kits for High Sensitivity
- MasterAmp™ High Fidelity RT-PCR Kits
- MasterPure™ RNA Purification Kits
- MasterAmp™ *Tth* DNA Polymerase

5. Recommended Reaction Conditions

For first-strand cDNA synthesis, we recommend using 5-25 units of MMLV RT per microgram of RNA template. For best results, run a pilot reaction as described by Sambrook *et al.*[4] Dilutions of the MMLV RT stock can be made up in 1X MMLV RT Buffer supplemented with 10 mM DTT. Dilutions should be made just prior to use and kept on ice.

First-strand cDNA Synthesis

1. Add the following reagents to a microcentrifuge tube at room temperature; add the MMLV RT last.

x	µl	RNase-free water
5	µl	10X MMLV RT Buffer
5	µl	0.1 M DTT
10	µl	dNTP Mix (2.5 mM each dNTP)
0.5	µg	oligo$(dT)_{12\text{-}18}$
1	µg	poly-A^+ selected mRNA
5-25	U	MMLV RT
50	µl	Total reaction volume

2. Incubate at 37°C for 60 minutes.

Actinomycin D can be used to inhibit synthesis of the second cDNA strand by MMLV RT.[3,4]

If used, actinomycin D should be added to final concentration of 50 µg/ml.

Warning: *Actinomycin D is a teratogen and a carcinogen.*

6. References

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