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Product Information

Version 1.2(ds), Revision 2013-01-25

Product Name	M-MLV Reverse Transcriptase
Code	A1111
Concentration	200 U/μl
Supplied with	5x first-strand Buffer
Store at	-20 ℃
Description	Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. This enzyme is isolated from *E. coli* expressing a portion of the pol gene of M-MLV on a plasmid. The enzyme is used to synthesize first-strand cDNA up to 5 kb.
Applications	Generation of first strand cDNA for use in: • PCR, see Protocol for First-strand cDNA Synthesis; • Second strand cDNA synthesis. • DNA labeling. • Real-time PCR; • Analysis of RNA by primer extension.
Unit Definition	One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37° C using poly(A)• oligo(dT) 25 as template-primer.
Storage Buffer	20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% NP-40, 50% glycerol
5 x First-strand Buffer	250mM Tris-HCl (pH 8.3 at 25℃), 375mM KCl,15mM $MgCl_2$, 50mM DTT
Quality Control Assay	This product has passed the following quality control assays: SDS polyacrylamide gel analysis for purity; functional absence of endodeoxyribonuclease, 3′ and 5′ exodeoxyribonuclease, and ribonuclease activities, yield and length of cDNA product.
Functional Assay	M-MLV Reverse Transcriptase was tested for use in the first strand cDNA synthesis

**** For laboratory research purpose and/or in vitro use only, and it is not to be used in humans or animals.***

Protocol

I. First-Strand cDNA Synthesis Using M-MLV RT

A 20-µl reaction volume can be used for 1ng–5µg of total RNA or 1–500ng of mRNA.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	poly(A) mRNA or specific RNA	1 to 500ng 1-5 µg
Prime	oligo $(dT)_{15}$ primer(50µM) or Random hexamer primer(50µM)	1 µl 1 µl
DEPC-treated water		to 13.4 µl
Total volume		13.4 µl

2. Mix gently, centrifuge briefly and incubate at 70 ℃ for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Prepare the following cDNA Synthesis Mix, add the following components in the indicated order:

5x first-strand buffer	4 µl
dNTPs (10 mM each)	1 µl
RNasin (40U/µl)	0.6 µl
M-MLV	1 µl

4. Mix gently and centrifuge

5. For oligo$(dT)_{15}$, incubate for 60 min at 42 ℃. For random hexamer primed synthesis, incubate for 60 min at 37 ℃.

6. Terminate the reaction by heating at 70 ℃ for 5 min.

The reverse transcription reaction product can be used immediately in second strand cDNA synthesis reactions or stored at -20° C for less than a week. For longer storage, -70°C is recommended.

II. PCR Reaction

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2µl of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50µl total volume.