Product Information

M-MLV Reverse Transcriptase

Catalog No.: 40511
Product Name: M-MLV Reverse Transcriptase

Description

M-MLV Reverse Transcriptase is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template with hybridized primer. In this product, a point mutation in the RNase H domain increases the thermostablity and provide higher cDNA yield of full-length transcripts than wild type M-MLV Reverse Transcriptase.

Unit definition

One unit of M-MLV Reverse Transcriptase incorporates 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C.

Concentration	Package size	Storage
100 U/μL	5 KU/set	-20°C

Storage buffer

20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.25 mM EDTA, 0.01% NP-40(v/v), 2.5 mM DTT, 50% glycerol (v/v).

5X RT Buffer

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

Features

- **Lack RNase H activity:** Weak RNaseH activity. High yield of long length cDNA.
- **Free from Endonuclease/Exonuclease activity**

Recommended Reaction Conditions:

The first-strand cDNA synthesis

1) Add the following reagents to a RNase free PCR tube on ice.

Oligo dT_{12-18} (1 μg) or random primer (50-250 ng) or gene-specific primer (2 pmole)	x μL
Total RNA (0.1-5 μg) or mRNA (0.1-0.5 μg) or unique RNA (0.01 pg - 0.5 μg)	x μL
DEPC ddH_2O	to 11 μL

2) Gently mix and incubate 5 min at 70°C then chill on ice for 2-10 min.
3) Centrifuge for few seconds then add the next composition on ice:

5X RT Buffer	4 μL
RNase Inhibitor (10 U/μL)	1 μL
dNTP Mix (10 mM each; Cat. No. 40701)	1 μL
DEPC ddH_2O	to 19 μL

4) Gently mix and incubate at 37°C for 5 min (for Oligo dT_{12-18} or gene-specific primer) or at 25°C for 5 min (for random primer).
5) Centrifuge for seconds before adding 1 μL M-MLV Reverse Transcriptase (100 U/μL).
6) Incubate at 42°C for 60 min (if use a random primer, incubate in 25°C for 10 min then incubate at 42°C for 60 min)
7) Inactivate at 70°C for 10 min.

For research use only.

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