PRODUCT INFORMATION

M-MLV RTase (M-MLV Reverse Transcriptase)

v. 230101

Catalog number

C15021-20000U / C15021-50000U

Package & Component

Cat.	Name	Amount
C15021-20000U	M-MLV Reverse Transcriptase (200 U/μL)	20,000 U
	5X M-MLV Reverse Transcriptase Reaction Buffer	1 mL
C15021-50000U	M-MLV Reverse Transcriptase (200 U/μL)	50,000 U
	5X M-MLV Reverse Transcriptase Reaction Buffer	1 mL

Description

Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase is an RNA-dependent DNA polymerase that synthesizes the first strand of complementary cDNA from a single-stranded RNA template with hybridized primer. This kit features high activity formulation of M-MLV RT and 5X reverse transcription buffer which are capable of full-length cDNA synthesis and high cDNA yields.

Source

Escherichia coli

Purity

>98% as determined by SDS-PAGE (purified by Ni-NTA chromatography).

Unit Definition

One unit is defined as the amount of the enzyme incorporates 1 nmol of dTTP into acid-insoluble product in 10 minutes at 37°C.

Reaction Condition

1X M-MLV Reverse Transcriptase Buffer, supplemented with dNTP mix, template and primer, Incubate at 37°C for synthesis of first strand cDNA.

5X M-MLV Reverse Transcriptase Buffer: 250 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$, 375 mM KCl, and 50 mM DTT.

Storage Buffer

M-MLV Reverse Transcriptase is supplied in 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).

Storage

Stored at -20°C. Avoid repeated freeze/thaw cycles.

Manuel

First strand cDNA synthesis:

1. Place all required reagents to a nuclease-free microcentrifuge tube and following the order suggested below.

Component	Amount	Final concentration
Oligo (dT)12-18 (50 μM) or random primer mix (60 μM)	1 μL	-
Total RNA template	X μL	1 μg
Nuclease-Free H_2O	Y μL	-
Total reaction volume	10 μL	-

2. Heat the tube to 65°C for 10 minutes to denature the secondary structure within RNA template. Immediately cool the tube on ice for 1 minute and centrifuge briefly in microcentrifuge. Add the following components to the annealed primer/RNA template, prepare on ice.

Component	Amount	Final concentration
5X Reverse Transcriptase Reaction Buffer	4 μL	1X
10 mM dNTPs mix	1 μL	0.5 mM each
RNase Inhibitor	X μL	20 U/rxn
M-MLV RTase	1 μL	200 U/rxn
Nuclease-Free H_2O	Y μL	-
Total reaction volume	20 μL	-

3. Incubate at 37°C for 1 hour. The extension temperature may be adjusted from 37°C to 42°C.
4. Inactivate the reaction at 65°C for 20 minutes. The cDNA products should be store at -20°C.
5. Reaction preparations may be scaled up or down proportionately.

Notes

After the reaction is complete, M-MLV RTase can be inactivated by incubation at 65°C for 20 minutes.

For Research Use Only.