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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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M-MLV RTase (M-MLV Reverse Transcriptase)

Cat no. LDG0006RF

Product Overview

Package component

Specification	Item	Amount
20,000 U	M-MLV Reverse Transcriptase	1 vial (200 U/μL)
	5X M-MLV Reverse Transcriptase Reaction Buffer	1 vial (1 mL)
50,000 U	M-MLV Reverse Transcriptase	1 vial (200 U/μL)
	5X M-MLV Reverse Transcriptase Reaction Buffer	1 vial (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

Activity

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

Storage buffer

The enzyme 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).

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 and Stability

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

Procedure

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 L	

3. Incubate at 37°C for 1 hour. The extension temperature may be adjusted from 37°C to 42°C.

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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.

Important notes

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

Disclaimer

This product is for research use only and is not intended for diagnostic use.

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