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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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M-MLV Reverse Transcriptase (Glycerol-Free)

Cat no. LDG0011RF

Product Overview

Package component

Specification	Item	Amount
20,000 U	M-MLV Reverse Transcriptase (Glycerol-Free)	1 vial (200 U/μL)
50,000 U	M-MLV Reverse Transcriptase (Glycerol-Free)	1 vial (200 U/μL)

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 enzyme dilution buffer. The enzyme formulation does not contain glycerol and is compatible for further lyophilization process.

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 and Stability

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

Procedure

First strand cDNA synthesis:

The following procedure is a general guideline for RT-qPCR reaction. To maintain an RNase-free environment, always wear disposable gloves, and use laboratory consumables and water of nuclease-free grade during the whole experiment course.

RT-qPCR reaction set-up:

1. Place all required reagents on ice.

Component	Amount	Final concentration
2X Probe qPCR Master Mix	10 μL	1X
M-MLV Reverse Transcriptase (Glycerol-Free)	1 μL	200 U/rxn
Forward primer (10 μM)	0.8 μL	0.4 μM
Reverse primer (10 μM)	0.8 μL	0.4 μM
Probe (10 μM)	0.4 μL	0.2 μM
RNA template	X μL	≦ 1 μg (total RNA)
Nuclease-Free H2O	Y μL	-
Total reaction volume	20 μL	-

2. Gently mix the reaction thoroughly to achieve uniform distribution and briefly centrifuge.
3. Thermal cycling conditions for standard qPCR

Step	Cycles	Temperature	Time
Reverse transcription	1	42 – 50°C	10 – 15 min
Enzyme activation	1	95°C	5 min
Denaturation	40 - 45	95°C	5 – 15 sec
Annealing/Extension		55 – 65 °C	30 – 60 sec

Important notes

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

Primer/Probe concentration

Final concentrations of 400 nM (each primer) are suitable for most reactions. To obtain optimal condition, primer

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concentration can be titrated between 0.2 – 1 µM.

A final concentration of 200 nM (probe) is suitable for most reactions. To obtain optimalcondition, probe concentration can be titrated between 0.1 – 0.3 µM.

Annealing/Extension optimization

To obtain optimal condition, annealing/extension temperature can be adjusted between 55°C – 65°C, annealing/extension time can be extended up to 60 sec.

Target length

Appropriate amplicon length should be arranged between 80 – 200 bp.

Disclaimer

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

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