PRODUCT INFORMATION

M-MLV Reverse Transcriptase (Glycerol-Free)

v. 230101

Catalog number

C15032-20000U / C15032-50000U

Package & Component

Cat.	Name	Amount
C15032-20000U	M-MLV Reverse Transcriptase (Glycerol-Free) (200 U/μL)	20,000 U
C15032-50000U	M-MLV Reverse Transcriptase (Glycerol-Free) (200 U/μL)	50,000 U

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

Purity

>98% as determined by SDS-PAGE analysis (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.

Storage

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

Manuel

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 H_2O	Y μL	-
Total reaction volume	20 μL	-

* See Usage Notes for additional guidelines on primer/template preparation.

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

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 concentration can be titrated between 0.2–1 μM.

A final concentration of 200 nM (probe) is suitable for most reactions. To obtain optimal condition, 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.

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