PR1MA™ Reverse Transcriptase, RNase H -

Reverse Transcriptase / Long cDNA synthesis

Contents

PR1MA™ reverse transcriptase, RNase H- is provided at a concentration of 200 U/uL with 10X reaction buffer.

Background

PR1MA™ reverse transcriptase, RNase H- is engineered to reduce RNase H activity and provide increased thermal stability. Reverse transcriptase, RNase H- can synthesize cDNA at a temperature optimum of 42˚C, providing increased specificity, higher yields of cDNA, and more full-length product. Because reverse transcriptase, RNase H- is not significantly inhibited by ribosomal and transfer RNA, it can be used to synthesize cDNA from total RNA.

Features

- Thermostability – between 40 - 50˚C with optimal activity at 42˚C
- Length of cDNA – can be used to synthesize first-strand cDNA up to 7 kb
- Applications – synthesis of first-strand cDNA, primer extension, sequencing dsDNA,
- cDNA libraries, RT-PCR, and RT LAMP

Application Notes

PR1MA™ reverse transcriptase, RNase H- can be used for first strand synthesis of complementary DNA (cDNA) from RNA or single-stranded DNA templates. It can be used with RT-qPCR assays, RT-LAMP, or cDNA library construction.

*These products are intended for research use only, not for diagnostic use. The safety and efficacy of these products in diagnostic or other clinical uses has not been established.

Shipping & Storage

- PR1MA™ reverse transcriptase, RNase H- is stored at -20˚C in 50% glycerol, 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 1 mM EDTA, pH 7.5. Can be supplied in a glycerol-free buffer as a custom order.
- PR1MA™ reverse transcriptase, RNase H- is shipped on dry or blue ice. On arrival store at -20˚C for optimum stability. Repeated freeze/thaw cycles should be avoided.

PR1MA™

Quality Control

- PR1MA™ reverse transcriptase, RNase H- Unit activity: A known reverse transcriptase is used to create a standard curve with a reverse-transcription quantitative PCR assay against which the activity of this enzyme is measured.
- Purity: >95% as determined by SDS-PAGE analysis
- PR1MA™ reverse transcriptase, RNase H- is free of detectable RNase and DNase (exo- and endonuclease).
- <0.05 ng contaminating host DNA per 200 U.

1X RT Reaction Buffer	3 mM MgCl 2
50 mM Tris-HCl	10 mM DTT
75 mM KCl	pH 8.3 at 25˚C

General Protocol for First Strand Synthesis of cDNA

1. In RNase- and DNase-free PCR tubes mix:

Component	Volume
RNA (1 ng - 5 ug)	n uL
10 mM dNTP mix	1 uL
50 uM Oligo(dT)$_{12-18}$ or 60 uM gene-specific primer	2 uL
Nuclease-free water	To 10 uL

2. Incubate the RNA/primer mixture at 65˚C for 5 minutes, then place on ice or 4˚C
3. Then add the following components

Component	Volume (1X)
10X Reaction Buffer	2 uL
RT (200 U/uL)	1 uL
RNase Inhibitor (40 U/uL)	0.2 uL
Nuclease-Free water	6.8 uL

1) Incubate the reaction mix at at 42˚C for 60 minutes.
2) Terminate reaction by incubating at 70˚C for 15 minutes.
3) Cool reaction on ice or at 4˚C.
4) Collect reaction via centrifugation.
5) cDNA can be stored at -20˚C or used immediately for PCR. The cDNA product should not exceed 1/10th of the PCR reaction volume.