PR1MA™ Reverse Transcriptase

An RNA-dependent DNA polymerase

PR1MA™ Reverse Transcriptase is an RNA-dependent DNA polymerase that can be used for complementary DNA (cDNA) synthesis from an RNA template and is ideal for use in PCR and isothermal amplification. Thunder RT is a robust enzyme that works in a broad range of temperatures (40 - 72˚C) and has RNase H activity.

Properties

- Optimal temperature: 55˚C
- Heat inactivation: 80˚C for 10 minutes
- 10X Isothermal buffer included. Please use supplied buffer for optimal results.
- Storage temperature: -20˚C
- Can be supplied in a glycerol-free/custom buffer.

Shipping & Storage

- RT is stored at -20˚C in 50% glycerol, 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, pH 7.5.
- Shipped on dry or blue ice. On arrival store at -20˚C for optimum stability.

We also offer a variety of encapsulated RNA controls for common targets and would love to work with you to develop a unique control to suit your needs. Contact us at tech@midsci.com

**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.*

Contents

PR1MA™ Reverse Transcriptase is provided at a concentration of 150 U/uL with 10X Isothermal buffer.

Background

Reverse transcriptase is an RNA-dependent DNA polymerase that can be used for complementary DNA (cDNA) synthesis from an RNA or DNA template and is ideal for use in RT-loop-mediated isothermal amplification (RT-LAMP). RT is a robust enzyme that works in a broad range of temperatures (40 - 72˚C) and has RNase H activity.

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Application Notes

RT is a robust enzyme used for first-strand synthesis of complementary DNA (cDNA) from RNA or single-stranded DNA templates. It is ideally suited for RT-loop-mediated isothermal amplification (LAMP) assays.

Quality Control

- RT Unit activity: A known reverse transcriptase is used to create a standard curve with a real-time qRT-PCR assay against which the activity of this enzyme is measured.
- Purity: >95% as determined by SDS-PAGE analysis
- PR1MA™ RT is free of detectable RNase and DNase (exo- and endonuclease).
- <0.05 ng contaminating host DNA per 15 U

1X Isothermal Reaction Buffer
50 mM Tris-HCl
75 mM KCl
3 mM $MgCl_2$
10 mM DTT
pH 8.3 at 25˚C

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General Protocol for LAMP Reaction

LAMP Reaction Mix:

Component	Stock	Volume	Final Concentration
10x Isothermal Buffer	10X	2.5 uL	1X
$MgSO_4$	100 mM	1 uL	4 mM
dNTP Mix	25 mM	1.25 uL	1.25 mM
Dye (Optional)	Variable	Variable	Variable
Primer mix	20X	1.25 uL	1X
Bts Polymerase	8 U/uL	1 uL	0.32 U/uL
Rnase Inhibitor	40 U/uL	1 uL	1.6 U/uL
RT	150 U/uL	0.1 uL	0.6 U/uL
Template RNA	Variable	Variable	Variable
Nuclease-free water		to 25 uL	
Final		25 uL	

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Laboratory Equipment & Supplies

Prepare mix in a clean, nuclease-free microcentrifuge tube and incubate at 64 - 72˚C for 30 min.

1. 10X Isothermal Buffer contains 20 mM $MgSO_4$ (2 mM per rxn); we recommend adding 4 mM $MgSO_4$ (on top of the 2 mM $MgSO_4$ contributed by the 10X Isothermal Buffer) to start and optimize assay
2. We recommend adding 4 mM $MgSO_4$ (on top of the 2 mM $MgSO_4$ contributed by the 10X Isothermal buffer) to start and optimize your assay from there
3. Intercalating dye (such as SYTO-82, SYTO-9, EvaGreen) are recommended for real time monitoring of amplification in LAMP reactions
4. A LAMP primer mix can be prepared with all 4 or 6 (with Loop) primers. A 20X primer mix should contain: 31.2 uM FIP, 31.2 uM BIP, 4 uM F3, 4 uM B3, 15.6 uM LoopF, 15.6 uM LoopB in TE or water
5. Thunder is provided at a concentration of 150 U/uL and the enzyme can be diluted into the reaction buffer to a lower concentration based on the user's needs. We recommend 3.75 U of enzyme per reaction
6. 1 ng-1 ug total RNA

Typical cDNA Synthesis Protocol

Component	Stock	Volume	Final Concentration
Template RNA	Variable	Variable	up to 1 ug
Isothermal Buffer	10X	2.5 uL	1X
50 uM Oligo(dT)$_{12-18}$ or 60 uM gene-specific primer	Variable	2.5 uL	5 or 6 uM respectively
dNTP mix	10 mM	1.25 uL	0.5 mM
RNase Inhibitor	40 U/uL	0.625 uL	1 U/uL
RT	150 U/uL	0.025 uL	0.15 U/uL
Nuclease-free water		to 25 uL	
Final		25 uL	

- Combine components described in the above table
- Incubate for 5 minutes at 25˚C for primer annealing
- Incubate between 40 - 72˚C for 20 minutes for cDNA synthesis
- Heat inactive at 80˚C for 10 minutes

PR1MA™ Reverse Transcriptase is provided at a concentration of 150 U/uL. The enzyme can be diluted into the reaction buffer for a lower concentration. Higher or lower amounts of enzyme can be used based on needs. We recommend starting at 15 U per LAMP reaction and 3.75 U per cDNA synthesis reaction.