Varizymes®
OPTIONS AMPLIFIED

# Thunder™ RT

## An RNA-dependent DNA polymerase

**Thunder 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

Thunder 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.

Varizymes also offers a variety of encapsulated RNA controls **VariSafe™** for common targets, and we would love to work with you to develop a unique control to suit your needs. For more information visit **www.varizymes.com** or email us at **info@varizymes.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.*

# Thunder reverse transcriptase

**Catalog No.: 7100**

## Contents

Thunder reverse transcriptase is provided at a concentration of 150 U/µL with 10X Isothermal buffer

## Background

Thunder 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). Thunder RT is a robust enzyme that works in a broad range of temperatures (40 - 72 °C) and has RNase H activity.

## Application Notes

Thunder 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

- **Thunder 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
- Thunder 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

# General Protocol for LAMP Reaction

## LAMP Reaction Mix

| Component | Stock | Volume | Final Concentration |
|---|---|---|---|
| [1]10X Isothermal Buffer | 10x | 2.5 µL | 1x |
| [2]$MgSO_4$ | 100 mM | 1 µL | 4 mM |
| dNTP Mix | 25 mM | 1.25 µL | 1.25 mM |
| [3]Dye (optional) | Variable | Variable | Variable |
| [4]Primer mix | 20x | 1.25 µL | 1x |
| *neo*Bolt™ Bst polymerase | 8 U/µL | 1 µL | 0.32 U/µL |
| RNase Inhibitor | 40 U/µL | 1 µL | 1.6 U/µL |
| [5]Thunder™ RT | 150 U/µL | 0.1 µL | 0.6 U/µL |
| [6]Template RNA | Variable | Variable | Variable |
| Nuclease-Free Water | | to 25 µL | |

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 $\mu$M FIP, 31.2 $\mu$M BIP, 4 $\mu$M F3, 4 $\mu$M B3, 15.6 $\mu$M LoopF, 15.6 $\mu$M LoopB in TE or water

[5]Thunder is provided at a concentration of 150 U/µL 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 µg total RNA

## Typical cDNA Synthesis Protocol

| Component | Stock | Volume | Final Concentration |
|---|---|---|---|
| Template RNA | Variable | Variable | up to 1 µg |
| Isothermal Buffer | 10x | 2.5 µL | 1x |
| 50 µM $Oligo(dT)_{12-18}$ or 60 µM gene-specific primer | Variable | 2.5 µL | 5 or 6 µM respectively |
| dNTP Mix | 10 mM | 1.25 | 0.5 mM |
| RNase Inhibitor | 40 U/µL | 0.625 µL | 1 U/µL |
| [1]Thunder™ RT | 150 U/µL | 0.025 µL | 0.15 U/µL |
| Nuclease-free water | | to 25 µL | |
| Final | | 25 µL | |

- 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

[1]Thunder is provided at a concentration of 150 U/µL. 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.