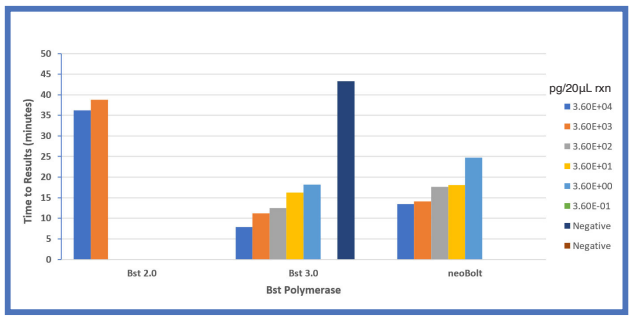
Varizymes®
OPTIONS AMPLIFIED

*neo*Bolt™ Bst Polymerase

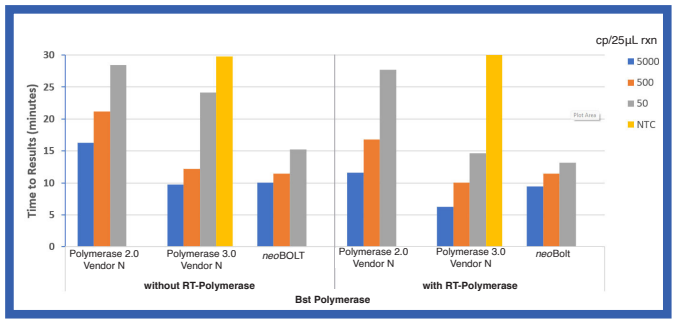
Recombinant, thermostable DNA polymerase for isothermal amplification

***neo*Bolt™ Bst polymerase** is a true single-enzyme reagent for developing RT-LAMP assays and is available either in glycerol or in a glycerol-free buffer as a custom order.

*neo*Bolt DNA LAMP



*neo*Bolt RT-LAMP



Bst Polymerase 2.0 and 3.0 are commercially available

Properties

- Only Bst Polymerase on the market with robust RT and DNA polymerase activity
- Thermostable, working temperature range 64 - 72 °C
- Tolerant to inhibitors
- Use *neo*Bolt Bst polymerase to develop LAMP assays with high sensitivity and specificity

Varizymes also offers a variety of encapsulated RNA controls for common targets, and we would love to work with you to develop a unique control to suit your needs. For more information visit or email **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.*

*neo*Bolt Bst Polymerase

Catalog No.: 9100

Contents

***neo*Bolt Bst polymerase** is provided at a concentration of 8 U/µL with 10X Isothermal buffer.

Background

***neo*Bolt Bst polymerase** is a recombinant, truncated (lacks 5' to 3' exonuclease activity), thermostable *Bacillus stearothermophilus* DNA polymerase with high reverse transcriptase and strand-displacement activities, ideal for isothermal amplification of RNA and DNA targets. ***neo*Bolt polymerase** is engineered to perform at temperatures up to 73 °C and tolerate inhibitors, has increased sensitivity and speed relative to other Bst polymerases, and can incorporate dUTP.

Application Notes

***neo*Bolt Bst polymerase** (exonuclease minus), with strong strand-displacement and RT activities can be used for amplification of DNA and RNA in loop-mediated isothermal amplification (LAMP).

Shipping and Storage

***neo*Bolt Bst polymerase** is supplied in a buffer of 50% glycerol, 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.05% Tween-20, 0.05% NP-40 substitute, pH=7.5.
Can be supplied in a glycerol-free buffer as a custom order.

Important note: use of the supplied buffer will yield optimal results.

***neo*Bolt Bst polymerase** is shipped on dry or blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided.

Quality Control

- ***neo*Bolt Bst polymerase** Unit activity: A known polymerase is used to create a standard curve with a real-time primer extension assay against which the activity of this enzyme is measured.
- Purity: >95% as determined by SDS-PAGE analysis
- ***neo*Bolt Bst polymerase** is free of detectable RNase and DNase (exo- and endonuclease).
- <0.05 ng contaminating host DNA per 8 U

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Setting Up LAMP Reaction

- Prior to setting up LAMP reaction, thaw all reaction components.
- Before use, mix all components by vortexing (5 sec) followed by centrifugation (5 sec).
- Setting up reaction on ice (4 °C) is highly recommended.

Reaction set up:

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	Variable	Variable	Variable
[4]Primer Mix	20x	1.25 µL	1x
*neo*Bolt™ Bst polymerase	8 U/µL	1 µL	0.32 U/µL
[5]RNase Inhibitor (optional)			
[6]RT polymerase (optional)			
Template	Variable	Variable	Variable
Nuclease-Free Water		to 25 µL	
Total			25

[1] 10X Isothermal Buffer contains 20 mM $MgSO_4$

[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 μM FIP, 31.2 μM BIP, 4 μM F3, 4 μM B3, 15.6 μM LoopF, 15.6 μM LoopB in TE or water

[5] **RNase Inhibitor** (#3001) recommended when using RNA target

[6] For RNA targets only. ***neo*Bolt polymerase** has strong RT activity, however, for faster time to results and increased sensitivity, the use of RT polymerase **Thunder RT** (#9000) is recommended

Recommended Companion Products

- VariSafe™ RNA Controls
- RNase Inhibitor
- MS2 Phage
- Cod Uracil-DNA glycosylase