NZY Bst DNA polymerase

Catalogue number: MB44401, 200 µL
MB44402, 3 x 200 µL

Description

NZY Bst DNA polymerase belongs to a new generation of *Geobacillus stearothermophilus* (formerly known as *Bacillus stearothermophilus*) DNA polymerases optimized for standard loop-mediated isothermal amplification (LAMP) applications. NZY Bst DNA polymerase displays 5´→3´ DNA polymerase activity together with a strong strand displacement activity, and thus lacks 5´→3´ exonuclease activity. The enzyme was engineered to produce robust LAMP amplifications in short running times, under minimal optimization conditions. NZY Bst DNA polymerase was engineered to provide high sensitivity, allowing the specific amplification of DNA fragments from low copy number templates. NZY Bst DNA polymerase is also suitable for nucleic acid amplification methods requiring strand displacement activity, such as Whole Genome Amplification (WGA) and Multiple Displacement Amplification (MDA).

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

All components should be stored at -20°C in a constant temperature freezer. Avoid multiple freeze-thaw cycles to guarantee maximal shelf life. The product will remain stable until the expiry date if stored as specified.

Pack components

Component	MB44401	MB44402
NZY Bst DNA polymerase (8 U/µL)	200 µL	3 x 200 µL
Reaction buffer, 10× (*)	2 x 500 µL	6 x 500 µL
Magnesium Sulfate ($MgSO_4$) solution (100 mM)	500 µL	3 x 500 µL

(*) Contains 20 mM $MgSO_4$ (leads to a final concentration of 2 mM Mg^{2+})

Unit definition

One unit is defined as the amount of enzyme required to catalyse the incorporation of 25 nmoles of dNTPs into acid insoluble material in 30 minutes at 63°C in controlled assay conditions.

Enzyme concentration 8 U/µL, in glycerol

Magnesium Sulfate solution

The provided 100 mM $MgSO_4$ solution allows users to optimize Mg^{2+} concentration in different amplification set-ups. For LAMP, NZY Bst DNA polymerase works effectively with 6 mM $MgSO_4$ concentration, but optimizations can be performed from 2 – 16 mM concentrations (NZY Bst DNA polymerase reaction buffer provides 2 mM $MgSO_4$ when 1x). Vortex the $MgSO_4$ solution thoroughly after thawing.

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for any LAMP application. Optimal reaction conditions (e.g. concentration of DNA polymerase, primers, $MgSO_4$ and template DNA) may vary, although optimization is usually not required. In case you need to fine-tune LAMP conditions, recommended variations of each PCR component are provided in brackets in the table below.

1. Gently mix and briefly centrifuge all components after thawing. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of LAMP reactions. Add water first and the remaining components in the order specified in the table below. A single reaction mixture of 25 µL should combine the following components:

Reaction buffer, 10×	2.5 µL
$MgSO_4$, 100 mM	6 (2-16) mM
dNTPs mix (not provided)	1.4 (1-1.8) mM
LAMP Primer Mix, 10x (*)	2.5 µL
Template DNA (see below)	> 100 copies
NZY Bst DNA polymerase (8 U/µL)	1 µL
Nuclease-free water	up to 25 µL

(*) For easy handling of multiple primers used in LAMP assay, we recommend preparing a mix 10x concentrated by combining all required primers. Recommendations for primer design and concentrations are described in section “Primer design” below.

2. Mix and quickly pulse the reaction.

3. Incubate at 63°C for 30 minutes. Time can be extended, and temperature can be adjusted (between 55˚C and 68˚C) as necessary for low copy templates or whenever amplification times have been previously reported as extensive.

Inactivation

NZY Bst DNA polymerase can be inactivated by heating at 80°C for 10 min.

Primer Design

A standard LAMP assay typically includes a set of 4 primers (2 outer primers and 2 inner primers) that will recognize distinct regions of the target gene. The four primers used are commonly designated as FIP (Forward Inner Primer), BIP (Backward Inner Primer), F3 (Forward Outer Primer), and B3 (Backward Outer Primer). Addition of two loop primers – LoopF (Forward Loop Primer) and LoopB (Backward Loop Primer) – allows to accelerate the isothermal reaction time and significantly promotes the LAMP reaction. A LAMP Primer Mix can be prepared with all 4 or 6 (if you include Loop primers). A 10x LAMP Primer Mix should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4-8 µM LoopF, and 4-8µM LoopB in TE Buffer or water.

General guidelines for the design of LAMP primers:

1. Length between 15 and 25 mer.
2. Amplicon length <300 bp and distance between FIP and BIP primers should be 120-160 bp.
3. GC content between 45-60%; be sure to avoid single or dinucleotide base repeats and secondary structure regions.
4. Melting temperature (T_m) should be similar across primer pairs (<5°C difference), allowing their annealing at roughly the same temperature.

DNA template

The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 10ng to 500ng of mammal genomic DNA templates.

Quality control assays

Purity

NZY Bst DNA polymerase purity is >90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

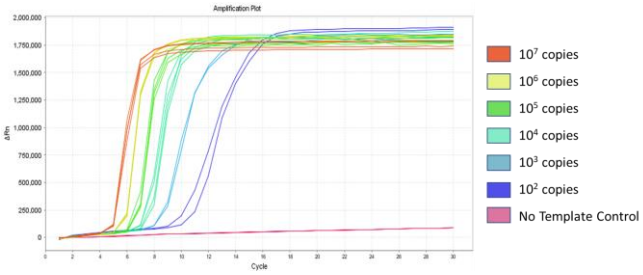
Genomic DNA contamination

NZY Bst DNA polymerase must be free of any detectable genomic DNA contamination as evaluated through PCR.

Nuclease assays

Typically, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 8 U of NZY Bst DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37°C. To test for RNase contamination, 1 µg of RNA is incubated with 8 U of NZY Bst DNA polymerase, in 1× Reaction Buffer for 1 h at 37°C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with the reaction buffer and $MgSO_4$ solution.

Functional assay



NZY Bst DNA polymerase is extensively tested for performance in real-time loop-mediated isothermal amplification (LAMP) assays. The Figure above represents a typical amplification plot of SARS-CoV-2 nucleic acids at different viral copy numbers in standard conditions (63°C).

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