Polaris® BstY Polymerase 8 U/µL

Catalogue number	Presentation
MD06781	5 mL (5k rxns of 25 µL)

Introducing the Polaris® brand

NZYtech, with its established expertise in enzyme development and IVD kit production, proudly introduces Polaris® - a groundbreaking series of newly developed diagnostic enzymes, master mixes and reagents. Polaris® brand products set the standard in purity, extreme stability, diagnostic performance, reliability, and regulatory compliance. These attributes are housed in functional packaging tailored for stringent laboratory applications. Polaris® stands at the forefront of innovation, designed to meet the complex demands of molecular diagnostics with a steadfast focus on quality and scientific integrity. At its core, Polaris® adheres to stringent international quality standards, including ISO 9001 and ISO 13485, ensuring its enzymes and reagents are perfectly suited for a wide array of IVD applications. These products surpass the stringent European IVDR requirements, demonstrating a commitment to quality management and excellence in every aspect of their development and production. Utilizing cutting-edge manufacturing protocols, precise control measures, and rigorous validation, Polaris® becomes the new benchmark for diagnostic testing. NZYtech's state-of-the-art facilities are optimized to produce these high-precision diagnostics tools, ensuring unmatched accuracy and performance. Our team is always ready to offer comprehensive support to our customers and partners, assisting with IVDR compliance and ensuring smooth integration, upon request. NZYtech is committed to advancing the field of molecular diagnostics, thereby expanding access to clinical results, enabling rapid diagnostics, and fostering research advancement.

Features

- Superior specificity at 65-70 °C, enhancing diagnostic accuracy and reducing non-specific amplifications.
- Powerful 5´→3´ DNA polymerase and strand displacement activities enable effective isothermal amplification.
- Extensively validated to deliver consistent performance across various conditions.

Description

Polaris® BstY Polymerase 8 U/µL represents the cutting edge in strand-displacing DNA polymerases, specifically optimized for loop-mediated isothermal amplification (LAMP) in diagnostic contexts. This enzyme exhibits potent 5´→3´ DNA polymerase activity, complemented by strong strand-displacement capabilities, without the 5´→3´ exonuclease activity. Designed for superior performance, it boasts an array of enhancements: unmatched resistance to inhibitors, exceptional robustness, rapid reaction kinetics, and superior thermostability. Engineered for high-temperature LAMP assays (optimal activity at 68-69 °C), Polaris® BstY Polymerase surpasses traditional enzymes by operating effectively at 65-70 °C. This not only increases the specificity of the assays by reducing non-specific amplifications but also mitigates the effects of inhibitors present in various sample types, essential traits for both clinical and research applications. NZYtech provides the Polaris® BstY enzyme in various formats to ensure versatility for end-users. This version is a robust glycerol-containing format. In summary, Polaris® BstY Polymerase 8 U/µL is rigorously validated and designed for adaptability, marking it as an essential tool for diagnostics. Its consistency and reliability extend across various biological matrices and assay conditions, meeting a wide range of diagnostic needs. For customers seeking to conduct a smaller scale of experiments, such as 200 reactions, we offer tailored enzyme packs designed to meet your specific needs (Polaris® LAMP Pack, Cat. No. MD0772).

Shipping & Storage Conditions

This product is shipped in dry ice. Upon arrival, promptly store all components at -85 °C to -15 °C in a constant temperature freezer. Avoid direct sunlight exposure. Immediately after use, return the components to a temperature between -85 °C and -15 °C to minimize exposure to room

temperature. This product is stable through a minimum of 10 freeze-thaw cycles. For users planning frequent use, it is advisable to aliquot the enzyme into smaller volumes to preserve its integrity. Adhering to these meticulous storage procedures ensures that Polaris® BstY Polymerase 8 U/µL will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

Components

COMPONENT		BOTTLE	VOLUME
BstY	Polaris® BstY Polymerase 8 U/µL	1	5 mL

Reagents, Materials and Equipment Required but Not Provided

To utilize Polaris® BstY Polymerase 8 U/µL in LAMP protocols, additional reagents are necessary, though not provided with this product. For optimal efficiency, we recommend the use of complementary reagents from NZYtech's innovative Polaris® series. Among the following recommended reagents are components such as the Polaris® BstY Reaction Buffer 10x – a standard reaction buffer for LAMP assays - and the Polaris® Lyophilizable LAMP Buffer 6x which is a more versatile and additivated reaction buffer compatible with lyophilization. Please note that the Polaris® Speedy LAMP Fluorescent dye 25x might not be required if not conducting a fluorescence-based evaluation of the amplification; however, this dye also contains several reaction accelerators that might be necessary in certain circumstances, even when fluorescence analysis is not performed (please refer to the technical note bellow – Signal Detection).

REAGENT NOT PROVIDED	TEST COMPONENT	CAT. NO.
Polaris® Lyophilizable LAMP Buffer 6x	**Lyo-able LAMP Buffer 6x**	MD0684
Polaris® BstY Reaction Buffer 10x	**BstY RxnBuffer 10x**	MD0688
Polaris® $MgSO_4$ 100 mM	**$MgSO_4$ 100 mM**	MD0689
Polaris® dNTPs mix 25 mM, IVD	**dNTP mix 25mM**	MD0690
Polaris® Speedy LAMP Fluorescent dye 25x	**Speedy LAMP dye 25x**	MD0756
DEPC-treated water	**DEPC-treated water**	MB43701

Other Essential Materials and Equipment required but not provided are:

- Real-time PCR Instrument (in case LAMP reaction is to be run in a thermocycler): Ensure the instrument is capable of detecting the FAM™/SYBR fluorescent dyes (emission wavelengths of approximately 520 nm).
- RNase & DNase-free PCR Plasticware: including PCR tubes, strips, caps, 96-well plates and adhesive films.
- Pipettors and Filter Tips: ensure that they are RNase & DNase-free.
- Disposable Gloves: to prevent contamination and maintain sample integrity.
- Vortex and Centrifuge: essential for mixing and reaction preparation.

Ensure that all reagents and equipment used comply with the appropriate standards for molecular diagnostic use. Follow all relevant guidelines and manufacturer recommendations for handling and use.

Standard Protocol

Recommendations before starting

- Handling instructions:
  - To help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up and LAMP amplification. It is essential that any tubes containing amplified product are not opened in the LAMP set-up area. Use sterile filtered tips.
  - All pipetting actions and experimental plate preparations must be diligently performed on benchtop coolers or ice to safeguard the integrity of the reagents and to mitigate the risk of generating LAMP artifacts, which could compromise the sensitivity and/or specificity of detection.
  - Upon plate preparation, swiftly progress to initiating the LAMP protocol; any delay or prolonged incubation of reaction mixes at room temperature may inadvertently foster the emergence of artifacts.
- Reagent usage:
  - It is strongly recommended to thoroughly review the usage instructions of all involved reagents before assay execution.
  - Ensure homogeneity of the reagents prior to use. To achieve this, gently flick the tubes provided to homogenise the contents, then centrifuge for a few seconds to collect the volume at the bottom of the tube. Maintain tubes on ice.
  - To avoid cross-contamination, we strongly recommend pipetting the template and particularly the Positive Controls last, only after all the other components have been used and remaining material properly stored.
- Controls: To verify the absence of contamination, prepare a negative control reaction without a template (No-template control or negative control). Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the LAMP reaction and detection system. The positive control should exhibit the expected amplification and/or fluorescence signal, confirming the assay's ability to accurately detect the target sequence.

Procedure for LAMP testing

This standard protocol provides a foundational guideline for conducting LAMP reactions. While it serves as a reliable starting point, some parameters may require adjustments based on specific needs, such as the reaction temperature or the initial quantity of template and, as such, this protocol can be adapted accordingly. The effectiveness of this suggested protocol is contingent on the proper storage and condition of all supplied components.

1. On ice, in a sterile nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (the volumes provided are to prepare a single reaction mixture of 25 µL):
   Notes:
   - *Add water first and the remaining components in the order specified in the table below.*
   - *When choosing the reaction buffer please take into consideration the characteristics described in section "Reagents, Materials and Equipment Required but Not Provided" above.*
   - *To calculate the total number of reactions required per assay, consider the total number of samples to test and include two additional reactions to accommodate the No-Template and Positive Controls.*
   - *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	TEST USING 10X BUFFER VOLUME (µL)	TEST USING 6X BUFFER VOLUME (µL)
Polaris® BstY Polymerase 8 U/µL (provided)	1	1
BstY RxnBuffer 10x	2.5	-
Lyo-able LAMP Buffer 6x	-	4.2
$MgSO_4$ 100 mM (*)	1.5	1.5
dNTP mix 25mM	1.4	1.4
LAMP Primer Mix, 10x (**)	2.5	2.5
Speedy LAMP dye 25x (***)	1	1
Template DNA	> 100 copies	> 100 copies
DEPC-treated water	**up to 25 µL**	**up to 25 µL**

(*) *Polaris® Buffers contain 2 mM $MgSO_4$, resulting in a combined final $MgSO_4$ concentration of 8 mM. Please consult the Technical Notes for further considerations regarding Mg^{2+} concentration.*
(**) *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 the Technical Notes below.*
(***) *Only required when aiming to follow the reaction by fluorescence-based real-time LAMP.*

2. Mix and quickly pulse the reaction.
3. Incubate at 69 °C for 20 minutes. Time can be extended up to 30 min, and temperature can be adjusted (between 65 ˚C to 70 ˚C) as necessary whenever amplification times have been previously reported as extensive.
   Note: *To assess the specificity of the LAMP amplification reaction when performing fluorescence-based real-time LAMP, it is highly recommended to include a Melt Curve step: 65 → 99 °C with 0.5 °C increments at 10 second intervals.*
4. Store at - 85 °C to -15 °C or directly proceed to downstream applications.

Technical notes

Sample material

Achieving optimal results in LAMP molecular testing requires meticulous attention to various factors, including the protocol for sample collection from biological specimens and the methods of sample transport, storage, and processing. Upon collection, samples should be promptly tested and must be transported and stored at low temperatures, complying with local biosafety regulations. Ensure the suitability of DNA/RNA samples in terms of purity, concentration, and nucleic acid integrity. The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 10 ng to 500 ng of mammal genomic DNA templates (>100 target copies/reaction).

Primers

A typical LAMP assay incorporates a set of four primers designed to specifically recognize distinct regions within the target sequence. These primers are categorized into two pairs: two outer primers and two inner primers, commonly referred to as FIP (Forward Inner Primer), BIP (Backward Inner Primer) for the inner pair, and F3 (Forward Outer Primer) and B3 (Backward Outer Primer) for the outer pair. To enhance the efficiency and reduce the reaction time of the isothermal amplification, an additional pair of loop primers, LoopF (Forward Loop Primer) and LoopB (Backward Loop Primer), can be incorporated. For the preparation of a LAMP Primer Mix, both sets comprising either 4 or 6 primers (including Loop primers) can be utilized. A recommended 10x LAMP Primer Mix should include the following concentrations: 16 µM each for FIP and BIP, 2 µM each for F3 and B3, and 4-8 µM each for LoopF and LoopB, diluted in TE Buffer or water. Please follow the general guidelines for LAMP Primer Design:

- Primer Length: Primers should be between 15 and 25 nucleotides in length.

- Amplicon Characteristics: Aim for an amplicon length of less than 300 base pairs, with the distance between the FIP and BIP primers ranging from 120 to 160 base pairs.
- GC Content: Maintain a GC content within the range of 45-60%. It is critical to avoid regions prone to forming secondary structures or containing single or dinucleotide repeats.
- Melting Temperature (Tm): Ensure that the melting temperatures of primer pairs are closely matched, with less than a 5°C difference, to facilitate uniform annealing across all primers.

Leveraging available online software for primer design is highly recommended, especially when targeting novel genes. These software tools are equipped with algorithms that consider the intricacies of primer design, including specificity, melting temperature (Tm), GC content, and potential for secondary structures or primer-dimer formation. For novel targets, it is advisable to design and evaluate multiple sets of primers to ensure optimal performance in LAMP assays. Using online software for primer design streamlines the process, making it more efficient and less prone to error. These tools can quickly analyze genetic sequences to identify optimal primer binding sites while minimizing the risk of non-specific amplification. Moreover, software can help predict potential problems such as primer-dimer formation or secondary structures, which are critical for the success of LAMP assays.

Testing multiple primer sets with control kits, such as those offered by NZYtech, NZY RT-LAMP Positive Control Kit (Cat. No. MB48101), or NZY RT-LAMP Positive Control Kit (Cat. No. MB48101), provides a systematic approach to selecting the best primer set, ultimately ensuring the success of molecular diagnostics applications. In addition, introducing a melting curve analysis is valuable for confirming the specificity and purity of the products generated during the LAMP reaction. A melt curve helps to distinguish between specific amplification products and non-specific products or primer dimers – specific products typically exhibit a sharp and distinct melting peak, corresponding to the expected Tm of the target sequence, while non-specific products or primer dimers may produce different melting temperatures or broad peaks, indicating less specificity.

$MgSO_4$

Mg^{2+} concentration optimization can be performed by testing a range between 2 – 16 mM of $MgSO_4$ final concentration. When using Polaris® Buffers for LAMP, it is essential to note that they already incorporate $MgSO_4$ in their formulation for a final (1x) concentration of 2 mM.

Signal Detection

The optional Polaris® Speedy LAMP Fluorescent Dye 25x (Cat. No. MD0756, not provided) is a DNA intercalating dye. As the amount of DNA increases during the amplification process, the dye intercalates into the DNA strands. This results in a fluorescent signal, which can be easily detected and quantified in real time. The intensity of the fluorescence is proportional to the amount of DNA amplified, providing a direct measure of the reaction's progress. Furthermore, this dye also contains reaction accelerators, which significantly reduce the time-to-result of a typical LAMP reaction. The combination of these characteristics makes the use of the Polaris® Speedy LAMP Fluorescent Dye 25x in a LAMP assay a powerful tool for the rapid, specific, and sensitive detection of nucleic acids. Polaris® BstY Reaction Buffer 10x leverages these principles to deliver reliable and efficient diagnostic results, making it an invaluable asset in molecular biology and diagnostic laboratories.

Negative Control

Including a negative control in LAMP assays is crucial to validate positive results and ensure the reliability of the assay. Due to the nature of LAMP reactions, which involve multiple primers, there is a significant risk of non-specific amplification, especially when the initial template amounts are low. A negative control, accompanied by melting curve analysis, is essential for identifying any non-specific amplification and serves as an internal validation of the assay's accuracy. To perform a negative control, simply replace the DNA template in the reaction mix with molecular-grade ultra-pure water. Since Polaris® BstY Reaction Buffer 10x can be combined with an engineered Polaris® Bst enzyme that operates at higher temperatures, the elevated amplification temperature minimizes the risk of non-specific amplification typically observed in these controls. In any case, melting curve analysis enables the differentiation of non-specific templates when compared to the target product.

Positive control

Including a positive control in each experiment is crucial to verify the assay's functionality. The absence of a signal in the positive control indicates a potential issue with the experimental setup, requiring further investigation and correction. If needed, NZYtech provides a LAMP Positive Control kit (Cat. No. MB48101), purposedly designed for validating LAMP reagents, set-ups, and reactions.

Data

A typical amplification plot obtained with Polaris® BstY Polymerase 8 U/µL and clinical samples containing viral nucleic acids is illustrated in Figure 1.

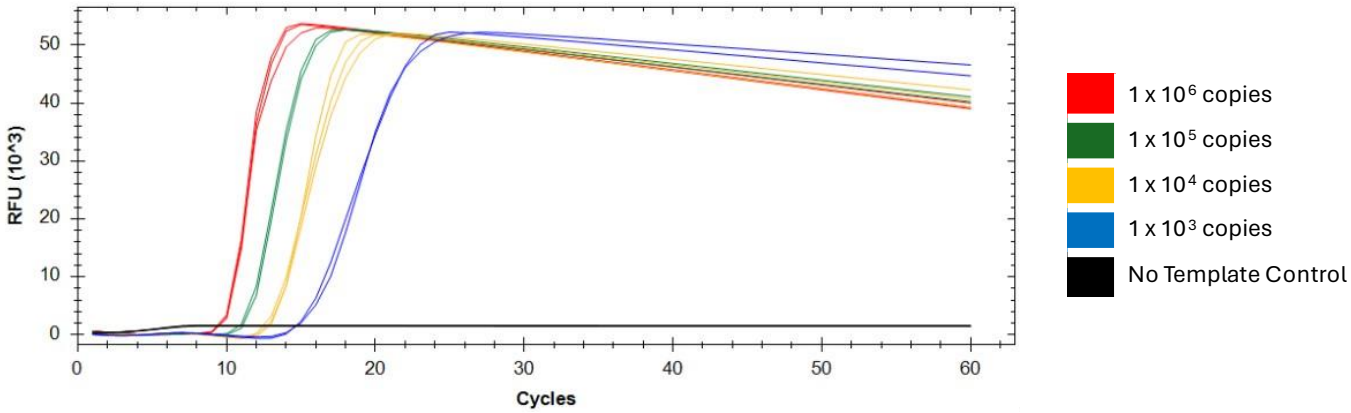

Figure 1. Typical LAMP Amplification Plot. The graph represents the amplification curves from a typical LAMP assay, displaying the real-time progression of DNA synthesis as measured by fluorescence intensity (ΔRn) on the y-axis against the number of cycles on the x-axis. The variety of colors corresponds to different template concentrations as described in the legend. The grey horizontal line at the lower part of the graph represents a no-template control (NTC), which shows no increase in fluorescence, thereby confirming the absence of contamination or non-specific amplification.

Quality control assays

Purity

Polaris® BstY Polymerase 8 U/µL is >98% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

Polaris® BstY Polymerase 8 U/µL is is tested to verify that ≤ 0.15 copies of bacterial gDNA and no human gDNA are present in a standard aliquot containing 1 unit of the enzyme. This is evaluated through qPCR detection.

Nucleases assay

No nuclease contamination is detected in Polaris® BstY Polymerase 8 U/µL given that there is no visible nicking or cutting of the nucleic acids. To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with 8 U of Polaris® BstY Polymerase 8 U/µL for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 8 U of the enzyme for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel.

Functional assay

Polaris® BstY Polymerase 8 U/µL is tested for performance in a LAMP experiment using a 10-fold serial dilution of gDNA (1 µg to 0.1 ng).

Troubleshooting

Troubleshooting LAMP assays requires a methodical approach, where altering one variable at a time and evaluating its impact can reveal the root cause of any issues encountered. The following recommendations are aimed at addressing common problems that may arise during LAMP amplification using Polaris® BstY Polymerase 8 U/µL. These adjusted suggestions, incorporating a blend of specific and exploratory approaches, aim to enhance the clarity and applicability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

NO AMPLIFICATION DETECTED
• **Inappropriate storage conditions**
Store Polaris® BstY Polymerase 8 U/µL away from light, preferably within the product box in a freezer (-85 °C to -15°C). Limit exposure to ambient laboratory lighting and avoid direct sunlight.
• **Excessive amount of sample in the reaction**
You may use up to 50% of the sample/template in a LAMP reaction. In the case of sample-caused reaction inhibition, this value should optimally be reduced to 4 to 10% per reaction, which corresponds to 1 to 2.5 µL of lysate per 25 µL reaction.

• **Suboptimal LAMP conditions**
Explore varying the reaction temperature, adjusting the reaction time, or increasing the amplification protocol over 20 minutes to enhance amplification. Ensure that these alterations do not result in non-specific product amplification.
• **Contamination with DNases**
Ensure that all labware, including pipettes, tubes, and containers, are clean and free from residual DNase/RNase contamination. Use DNase/RNase-free, autoclaved, or sterile equipment whenever possible. Use DNase/RNase-free water (we highly recommend using NZYtech's ultra-pure, DEPC-water – MB47301). Change gloves frequently.
LOW AMPLIFICATION YIELD
• **Ineffective reaction temperature**
Optimize the LAMP protocol by tweaking the reaction temperature and time, while observing the impact on amplicon yield.
• **Presence of inhibitors**
While robust to most inhibitors, Polaris® BstY Polymerase 8 U/µL is nevertheless susceptible to inhibition. Most common inhibitors are well tolerated in normal quantities, but larger amounts may reduce reaction efficiency and amplicon yield. Do not use overly crude samples in large amounts or less-than-ideal reaction components (for example, molecular-grade, DEPC-treated water is always recommended).
AMPLICON INCORRECT SIZE OR UNEXPECTED AMPLICON NUMBERS
• **Primer mis-design or mis-binding**
Verify the design of the primers and validate their ability to selectively amplify the desired fragment from 1-10 ng of purified DNA. Poor LAMP primer design is responsible for most non-specific amplifications.
• **Cross-contamination**
Create separate work areas for sample processing and LAMP setup. Use separate, dedicated pipettes, and disposable tips for each setup. Always follow good laboratory practices to avoid contamination. Include negative controls (no DNA template) in LAMP reactions to monitor for contamination in reagents or labware.

By systematically addressing these troubleshooting aspects, users can enhance the performance and reliability of LAMP assays conducted with Polaris® BstY Polymerase 8 U/µL. Remember that troubleshooting is a progressive process, and careful attention to detail can lead to resolutions. If persistent issues occur, please consult with NZYtech's technical support for further assistance.