Polaris® Lyophilizable LAMP Pack

Catalogue number	**Presentation**
MD07711	200 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

- Provides a full set of optimized reagents for comprehensive LAMP assay development and testing.
- Enhances assay precision at 65-70°C, reducing non-specific amplification.
- Includes reagents formulated for immediate lyophilization, simplifying storage and shipping.
- Offers Polaris® components rigorously tested for reliable performance in diverse diagnostic applications.

Description

The Polaris® Lyophilizable LAMP Pack is an all-encompassing kit designed to streamline loop-mediated isothermal amplification (LAMP) testing for scientists and diagnostic professionals. This comprehensive package is tailored to assess the functionality and performance of Polaris® reagents, enabling the exploration of both lyophilizable and non-lyophilizable LAMP solutions. At its core, the pack features the Polaris® Glycerol-free BstY Polymerase 80 U/µL, a next-generation enzyme that showcases exceptional 5´→3´ DNA polymerase and strand displacement activities, optimized for high-efficiency LAMP assays. Included within the pack are critical 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. Also included in the pack are the Dilution Buffer for Polaris® Glycerol-free BstY Polymerase, Polaris® dNTP mix 25mM, Polaris® $MgSO_4$ 100 mM, Polaris® Speedy LAMP Fluorescent dye 25x, and DEPC-treated Water. These elements are specifically chosen to allow users to tailor their LAMP assays, whether for direct application or for preparing lyophilized test formats, ensuring adaptability across a wide spectrum of diagnostic needs. The versatility of the Polaris® Lyophilizable LAMP Pack allows testing professionals to meticulously evaluate the performance of the Polaris® BstY Polymerase in conjunction with the efficacy of both 10x and 6x reaction buffers. This is particularly beneficial for those looking to optimize their assays for lyophilization or seeking to compare lyophilizable versus non-lyophilizable setups. In essence, the Polaris® Lyophilizable LAMP Pack not only facilitates a comprehensive assessment of Polaris® reagents in LAMP testing but also empowers users to fine-tune their protocols for maximal efficiency and effectiveness. Whether the goal is to explore the limits of Polaris® BstY Polymerase, to discern the optimal buffer conditions, or to develop stable, lyophilized test kits for field use, this pack serves as an essential toolkit. Rigorously validated and engineered for flexibility, the components of the Polaris® Lyophilizable LAMP Pack are indispensable allies in the pursuit of precise, reliable LAMP diagnostics across various conditions and sample matrices.

Shipping & Storage

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. Adhering to these meticulous storage procedures ensures that the Polaris® Lyophilizable LAMP Pack will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

Components

COMPONENT		TUBES	VOLUME
GF BstY 80 U/µL	Polaris® Glycerol-free BstY Polymerase 80 U/µL	1	20 µL
DBuffer for GF BstY	Dilution buffer for Polaris® Glycerol-free BstY Polymerase	1	500 µL
BstY RxnBuffer 10x	Polaris® BstY Reaction Buffer 10x	1	1 mL
Lyo-able LAMP Buffer 6x	Polaris® Lyophilizable LAMP Buffer 6x	1	1 mL
$MgSO_4$ 100 mM	Polaris® $MgSO_4$ 100 mM	1	1 mL
dNTP mix 25 mM	Polaris® dNTP mix 25 mM	1	300 µL
Speedy LAMP dye 25x	Polaris® Speedy LAMP Fluorescent dye 25x	1	200 µL
DEPC-treated Water	DEPC-treated Water	3	3 x 1 mL

Reagents, Materials and Equipment Required but Not Provided

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.
  - Use the DEPC-treated water provided. Alternatively, use a sterile molecular grade, nuclease free water.
- 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.

Procedures before starting

- It is advisable to perform a 1/10 dilution of GF BstY 80 U/µL with the provided Dilution Buffer for Polaris® Glycerol-free BstY Polymerase (DBuffer for GF BstY). When diluted, the quantity of enzyme added to the reaction must be adjusted according to the procedure outlined for LAMP testing below.

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 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 listed below 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. The template should be the last component added, preferably in a separate work area.*
- *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)
GF BstY 80 U/µL (*)	0.1	0.1
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 (not provided) (***)	2.5	2.5
Speedy LAMP dye 25x (****)	1	1
Template DNA (not provided)	> 100 copies	> 100 copies
DEPC-treated water	**up to 25 µL**	**up to 25 µL**

(*) *It is recommended to dilute the enzyme according to the instructions outlined in the "Procedures before starting" section. In such cases, use 1 µL of the diluted enzyme instead and be sure to adjust the volume of DEPC-treated water accordingly.*

(**) *Polaris® Buffers contain $MgSO_4$ to a final concentration of 2 mM. The addition of this extra volume of $MgSO_4$ results in a final magnesium concentration of 8 mM in the reaction. The final magnesium content can be adjusted to meet the requirements of specific reactions.*

(***) *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 reactions.

3. Run the general LAMP protocol defined below in a real-time instrument for fluorescence-based real-time LAMP:

NUMBER OF CYCLES	TEMPERATURE	TIME	STAGE
40	69 °C (*)	30 seconds	Amplification
1	95 °C	3 minutes	Enzyme inactivation
1	65 → 99 °C	10 second intervals	Melting curve (**)

(*) *Amplification* 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.

(**) *While not mandatory, the addition of a melting curve profile is invaluable for evaluating reaction efficiency and specificity* of the amplification reaction when performing fluorescence-based real-time LAMP.

4. Store at - 85 °C to -15 °C or directly proceed to downstream applications.

Lyophilization protocol

Procedure for preparing a lyophilized LAMP Master Mix

The Polaris® Lyophilizable LAMP Pack is specifically designed to generate 4x Lyo-able Master Mixes when the Polaris® Lyophilizable LAMP Buffer 6x is selected for master mix preparation. This buffer incorporates a proprietary blend of cryoprotectants and stabilizers, ensuring optimal performance and stability of the lyophilized product. The protocol outlined below describes the preparation and lyophilization of a 4x master mix, including the addition of GF BstY 80 U/µL and dNTPs, for efficient, robust LAMP testing. Theoretically the mix could also account for the inclusion of primers. Keep in mind that the efficiency and compatibility of primer inclusion should be validated on a case-by-case basis. The following is a suggestion for the preparation of a lyophilizable master mix 4x:

1. Thaw all components on ice. Before any tube is opened, vortex all of them before pulse-spinning their contents.
   Note: *Avoid using the same vortex and centrifuge used for the template with the other reaction components, particularly once they have been opened.*
2. In the clean reaction setup area, prepare a 4x LAMP Master Mix reaction mixture according to the table below that specifies the volumes for 1 and *n* reactions, as required for your experiments, into a sterile tube (not provided).

COMPONENT	1 REACTION VOLUME (µL)	*n* REACTIONS VOLUME (µL)
Lyo-able LAMP Buffer 6x	4.17	*n* x 4.17
dNTP mix 25mM	1.4	*n* x 1.4
GF BstY 80 U/µL	0.1	*n* x 0.1
DEPC-treated water	0.58	*n* x 0.58
TOTAL	**6.25**	***n* x 6.25**

3. Gently mix the solution by pipetting up and down or using a vortex mixer at a low speed to ensure thorough mixing of the components. Avoid introducing air bubbles to prevent any adverse effects on the lyophilization process.
4. Dispense the master mix into suitable lyophilization vials. The volume per vial will depend on the intended use and the scale of the assays. It is advisable to spin the vials before freezing.
5. Freeze the aliquots using a suitable method: a slow freezing process is ideal for adequate ice crystal formation however, if fast freezing is required to avoid precipitation or changes in pH, then an annealing process can be used (annealing the product by taking it up to -15 ºC, holding for 3 hours and allowing ice crystals to expand and grow from that point). The freezing step is critical to maintain the integrity of the components and facilitate a smooth lyophilization process.
6. Transfer the frozen aliquots to a lyophilizer (freeze-dryer). The lyophilization process should be carried out according to the equipment's specifications, typically involving a primary drying phase at low pressure and temperature, followed by a secondary drying phase to remove any bound water.

The following is a suggestion of a lyophilization protocol that has been validated for this 4x Master Mix, and could be changed to suit the desired outcome of the protocol, the equipment used, or other factors the user might deem important to modify:

PRESSURE	TEMPERATURE	TEMPERATURE RAMP	TIME	STAGE
Atmosferic	-40 °C (-35 → -60 °C)	N/A	4-6 hours	Primary freezing*
70 mTorr (50-100 mTorr)	-45 °C	0.3 °C/ min	2 hours	Primary drying, first stage
70 mTorr (50-100 mTorr)	- 40 °C	0.3 °C/ min	16-30 hours	Primary drying, second stage
70 mTorr (50-100 mTorr)	+25 °C	0.3 °C/ min	4-6 hours	Secondary drying

** If samples have been previously frozen outside of the equipment, skip this step. Make sure that the equipment's shelves are properly cooled before transferring samples onto them.*

Upon freeze-drying protocol completion, it is recommended that the equipment is filled with an inert gas (ex: N2) to avoid the introduction of water vapor whilst breaking the vacuum seal. To use, reconstitute the lyophilized master mix with a suitable volume of nuclease-free water or buffer, based on the desired final concentration for the LAMP assay.

The lyophilization protocol may require optimization based on the specific equipment and conditions in your laboratory. It is recommended to perform initial trials with small volumes to determine the optimal lyophilization and storage conditions for your specific applications. By following this comprehensive protocol, researchers and diagnostic professionals can prepare robust, stable, and efficient LAMP assays using the Polaris® Lyophilizable LAMP Buffer 6x, tailored for a wide range of diagnostic needs. If you require assistance in optimizing a lyophilization protocol adapted to your solutions, please get in touch with our technical support team (support@nzytech.com or info@nzytech.com).

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 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, these softwares can assist in predicting potential issues 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 (included in the pack) 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.

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® Lyophilizable LAMP Pack with samples containing viral nucleic acids is illustrated in Figure 1.

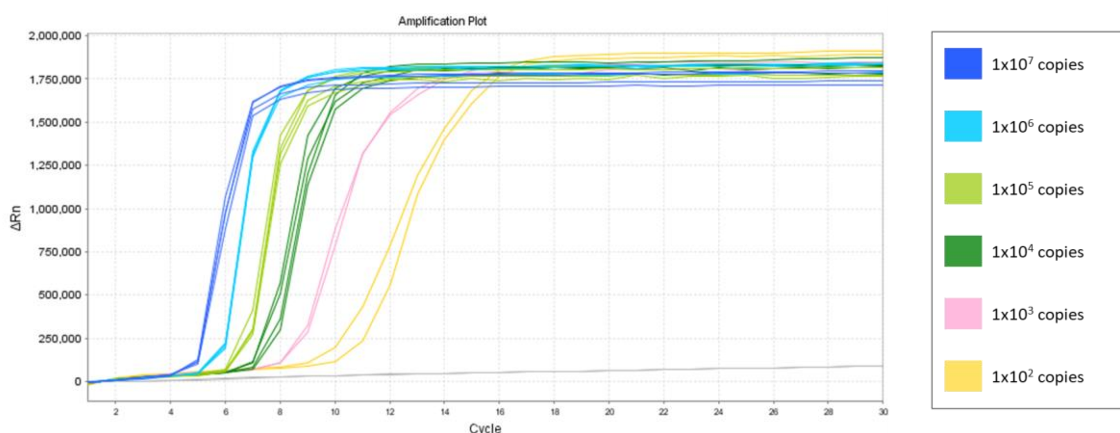

Figure 1. Typical LAMP Amplification Plot. The graph represents the amplification curves of a typical LAMP assay, displaying the real-time progression of DNA synthesis as measured by fluorescence intensity (ΔRn) on the y-axis against the number of cycles on the x-axis. The variety of colors corresponds 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

The enzyme included in the Polaris® Lyophilizable LAMP Pack is >98% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

Polaris® Lyophilizable LAMP Pack components are tested to verify that ≤ 0.15 copies of bacterial gDNA and no human gDNA are present. This is evaluated through qPCR detection.

Nucleases assay

No nuclease contamination is detected in Polaris® Lyophilizable LAMP Pack 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 each of the Polaris® Lyophilizable LAMP Pack components for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with these components for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel.

Functional assay

The components of Polaris® Lyophilizable LAMP Pack are extensively tested for performance in LAMP experiments.

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 the Polaris® Lyophilizable LAMP Pack. 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® Lyophilizable LAMP Pack 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 or adjusting reaction time, such as increasing the amplification protocol over 20 min 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, is 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 – MB43701). Change gloves frequently. If need to store extract samples for an extended period, always freezing them between -85 °C and -65 °C; this will help prevent DNase/RNase activity.
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, reactions using Polaris® Lyophilizable LAMP Pack are nevertheless susceptible to inhibition. Most common inhibitors are well tolerated in normal quantities, but larger amounts may reduce reaction efficiency and amplicon yield. Take care not to use overly crude samples in large amounts or less-than-ideal reaction components (for example, molecular-grade, DEPC-treated water is always recommended).
AMPLICON WITH 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® Lyophilizable LAMP Pack. 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.

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