Polaris® Lyophilizable RT-LAMP Buffer 6x

Catalogue number	Presentation
MD06851	21 mL (5k rxns of 25 µL)
MD06852	2 x 21 mL (10k 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 of products are unsurpassable in their purity, 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

- Optimized for High-Efficiency RT-LAMP Testing, enabling precise and robust amplification with enhanced specificity at operational temperatures of 65-70°C when combined with Polaris® BstY Polymerases and RTY Reverse Transcriptase.
- Specially Formulated for Lyophilization, offering superior stability through freeze-drying for extended storage and simplified shipping.
- Versatile Dilution Capability, allowing easy adjustment from 6x to 4x concentration to accommodate a wide range of RT-LAMP assay components.
- Compatible with fluorescent-based real-time RT-LAMP assays, free from any inhibitors that might interfere with such reactions.

Description

Polaris® Lyophilizable RT-LAMP Buffer 6x represents the forefront of reagent innovation for the development of robust, precise, and cost-efficient reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays. This buffer is expertly formulated to achieve the optimal balance of additives, stabilizers, and cryoprotectants, ensuring the production of highly efficient RT-LAMP mixtures. Designed for versatility, it allows for the dilution from a 6x to a 4x concentration through the addition of other necessary RT-LAMP components such as fluorescent dyes, dNTPs, enzymes, and primers. This adjustment results in a 4x reaction Master Mix that encompasses all essential constituents for RT-LAMP testing, excluding the template. Moreover, the buffer is uniquely tailored to support lyophilization, incorporating freeze-drying complementary ingredients to enhance stability during long-term storage and shipping, while also preserving efficacy in liquid form for direct use. The inclusion of such freeze-drying enhancer ingredients enables the creation of lyophilized or liquid master mixes, catering to a broad spectrum of diagnostic applications. Polaris® Lyophilizable RT-LAMP Buffer 6x was specifically designed to allow RT-LAMP reactions using thermostable Reverse Transcriptases such as Polaris® Glycerol Free Reverse Transcriptase Y 1000 U/µL, IVD (NZYtech, Cat. No. MD0674), Polaris® Glycerol-free Ribonuclease Inhibitor Y 160 U/µL, IVD (NZYtech, Cat. No. MD0691), and Bst DNA polymerase or other enzyme derivatives, such as Polaris® BstY Polymerase (NZYtech, Cat. No. MD0677). When combined with these highly specialized Polaris® enzymes, the Polaris® Lyophilizable RT-LAMP Buffer 6x transcends standard performance metrics, enabling robust RT-LAMP amplification at higher operational temperatures (65-

70 °C, with an optimal range of 68-69 °C). The elevated temperature range not only boosts assay specificity but also fortifies the master mix against inhibitors that frequently compromise assay integrity in both clinical and non-clinical samples. Polaris® Lyophilizable RT-LAMP Buffer 6x, through its sophisticated design and extensive validation, emerges as an indispensable asset for the development of RT-LAMP-based diagnostic tools. It ensures that the resulting master mixes meet the highest standards of reliability and consistency, across diverse biological matrices and assay conditions, thereby fulfilling a wide array of diagnostic requirements with unparalleled precision.

Shipping & Storage

This product is shipped in dry ice. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. Avoid direct sunlight exposure. Immediately after use, swiftly 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 Polaris® Lyophilizable RT-LAMP Buffer 6x will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

Components

This product provides the following set of reagents in sufficient amounts to perform 5000 or 10 000 *in vitro* RT-LAMP reactions of 25 µL each.

COMPONENT		CAT. NO.	TUBES	VOLUME
Lyo-able RT-LAMP Buffer 6x	Polaris® Lyophilizable RT-LAMP Buffer 6x	**MD06851**	1	21 mL
		MD06852	2	21 mL

Reagents, Materials and Equipment Required but Not Provided

To utilize Polaris® Lyophilizable RT-LAMP Buffer 6x in RT-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, particularly 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).

REAGENTS NOT PROVIDED	TEST COMPONENT	CAT. NO.
Polaris® Glycerol-free BstY Polymerase 80 U/µL	**GF BstY 80 U/µL**	MD0677
Polaris® Glycerol Free Reverse Transcriptase Y 1000 U/µL, IVD	**GF RTY 1000 U/µL**	MD0674
Polaris® Glycerol-free Ribonuclease Inhibitor Y 160 U/µL, IVD	**GF RIY 160 U/µL**	MD0691
Polaris® dNTPs mix 25 mM, IVD	**dNTP mix 25mM**	MD0690
Polaris® $MgSO_4$ 100 mM	**$MgSO_4$ 100 mM**	MD0689
Polaris® Speedy LAMP Fluorescent dye 25x*	**Speedy LAMP dye 25x***	MD0756
DEPC-treated water	**DEPC-treated water**	MB43701

*Only required if the RT-LAMP reaction is to be monitored in real-time through fluorescence in a specialized thermocycler.

Other Essential Materials and Equipment required but not provided are:

- Real-time PCR Instrument (in case RT-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 RNA/DNA contamination, you should assign independent areas for reaction set-up and RT-LAMP amplification. It is essential that any tubes containing amplified product should not be opened in the RT-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 RT-LAMP artifacts, which could compromise the sensitivity and/or specificity of detection.
- Upon reaction setup, swiftly progress to initiating the RT-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 contents 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.
  - Always use 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 RT-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 RT-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. Thaw all components on ice. Before any tube is opened, quickly 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 used.*

2. In the clean reaction setup area, prepare a RT-LAMP 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).
   Note: *Include sufficient reactions for the No-Template and positive controls, if required. We strongly recommend performing replicates of all reactions.*

COMPONENT	1 REACTION VOLUME (µL)	*n* REACTIONS VOLUME (µL)
Lyo-able RT-LAMP Buffer 6x	4.17	*n* x 4.17
Primer Mix 10x (not provided)	2.5	*n* x 2.5
$MgSO_4$ 100 mM (not provided) (*)	1.5	*n* x 1.5
Speedy LAMP dye 25x (not provided)	1	*n* x 1
dNTP mix 25mM (not provided)	1.4	*n* x 1.4
GF BstY 80 U/µL (not provided)	0.1	*n* x 0.1
GF RTY 1000 U/µL (not provided)	0.05	*n* x 0.05
GF RIY 160 U/µL (not provided)	0.25	*n* x 0.25
DEPC-treated water (not provided) (**)	9.03	*n* x 9.03
TOTAL	**20**	***n* x 20**

(*) The Lyo-able RT-LAMP Buffer 6x includes $MgSO_4$ for a final (1x) 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.
(**) If necessary, the water volume can be reduced to allow for a maximum template usage of 12.5 µL. In this instance, the volume is tailored for using 5 µL of template, although this amount may vary.

3. Pipette 20 µL of the RT-LAMP reaction mixture into individual wells, according to your RT-LAMP experimental plate/strip/tube configuration.
4. For the No-Template Control, add 5 µL of ultra-pure, molecular-grade water, instead of the RNA template, into the designated well(s). The final volume in each well should be 25 µL. Cover wells with appropriate caps.
5. For the remaining reactions, add up to 5 µL of your template into the respective wells (add molecular-grade water to fill up to 5 µL if needed). The final volume should be 25 µL.
6. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the RT-LAMP detection steps.
7. Place the reaction plate/strip/tube within the real-time instrument and run the general RT-LAMP protocol defined below. These conditions might be adapted to suit your specific needs, within sensible limits.

NUMBER OF CYCLES	TEMPERATURE	TIME	STAGE
40	69 °C (65-70 °C)	30 seconds	Amplification
1	95 °C	3 minutes	Enzyme inactivation
1	65 → 99 °C	10 second intervals	Melting curve *

***** *While not mandatory, the production of a Melting curve profile is invaluable when evaluating reaction efficiency and specificity.*

8. To assess the specificity of the RT-LAMP amplification reaction when performing fluorescence-based real-time RT-LAMP, it is highly recommended to include a Melt Curve step: 65 → 99 °C at 10 second intervals.
9. Store at - 85 °C to -15 °C or directly proceed to downstream applications.

Lyophilization Protocol

Procedure for preparing a lyophilized RT-LAMP Master Mix

The Polaris® Lyophilizable RT-LAMP Buffer 6x is specifically designed for seamless integration into lyophilization processes, facilitating the creation of stable, ready-to-use RT-LAMP assays. 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 enzymes, dNTPs, and potentially primers, for efficient, robust RT-LAMP testing. 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 lyophilzable master mix 4x.

1. Thaw all components on ice. Before any tube is opened, quickly 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 used.*

2. In the clean reaction setup area, prepare a 4x RT-LAMP Master Mix 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 25 mM (not provided)	1.4	*n* x 1.4
GF BstY 80 U/µL (not provided)	0.1	*n* x 0.1
GF RTY 1000 U/µL (not provided)	0.05	*n* x 0.05
GF RIY 160 U/µL (not provided)	0.25	*n* x 0.25
DEPC-treated water (not provided)	0.28	*n* x 0.28
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 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. Small aliquots are recommended to minimize the need for repeated freeze-thaw cycles post-lyophilization.
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: N_2) 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 RT-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 RT-LAMP assays using the Polaris® Lyophilizable RT-LAMP Buffer 6x, tailored for a wide range of diagnostic needs. If you require assistance in optimizing a lyophilization protocol tailored 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 RT-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 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 1 ng to 500 ng of mammal mRNA templates (>100 target copies/reaction).

Primers

A typical RT-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 RT-LAMP Primer Mix, both sets comprising either 4 or 6 primers (including Loop primers) can be utilized. A recommended 10x RT-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 RT-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 RT-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 software tools can assist in predicting potential issues such as primer-dimer formation or secondary structures, which are critical for the success of RT-LAMP assays.

Testing multiple primer sets with control kits, such as those offered by NZYtech, NZY LAMP Positive Control Kit (Cat. No. MB4801), 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 RT-LAMP reaction. A melt curve helps to distinguish between specific amplification products and nonspecific products or primer dimers – specific products typically exhibit a sharp and distinct melting peak, corresponding to the expected Tm of the target sequence, while nonspecific 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. It is essential to note that the Polaris® Lyophilizable RT-LAMP Buffer 6x already incorporates $MgSO_4$ in its formulation for a final (1x) concentration of 2 mM.

Signal Detection

The optional Polaris® Speedy LAMP Fluorescent Dye 25x (not included) 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 RT-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® Lyophilizable RT-LAMP Buffer 6x 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 RT-LAMP assays is crucial to validate positive results and ensure the reliability of the assay. Due to the nature of RT-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 (i.e. a reaction without template, or No-Template 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 RNA template in the reaction mix with molecular-grade ultra-pure water. Since Polaris® Lyophilizable RT-LAMP Buffer 6x is specifically designed for use with the newly developed class of Polaris® enzymes (that operate at higher temperatures), the elevated amplification temperature minimizes the risk of non-specific amplification typically observed in Non-Template 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 RT-LAMP Positive Control kit (MB481), purposedly designed for validating RT-LAMP reagents, set-ups, and reactions.

Data

Typical amplification plots obtained with Polaris® Lyophilizable RT-LAMP Buffer 6x with clinical samples containing viral nucleic acids are illustrated in Figure 1.

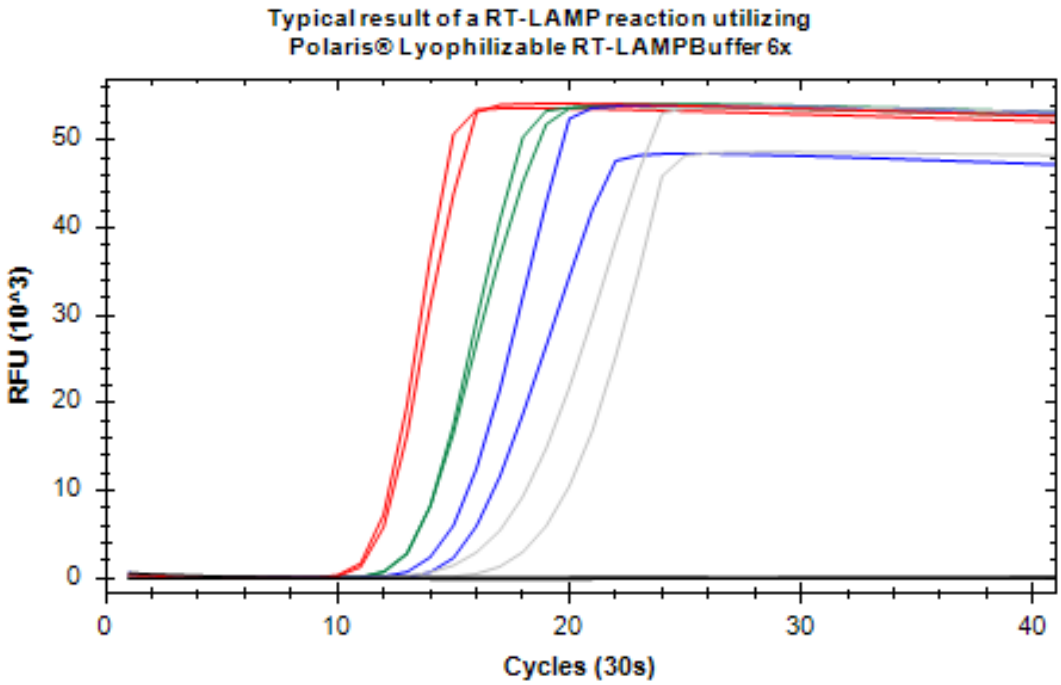

Figure 1. Typical RT-LAMP Amplification Plot. The graph represents the amplification curves of a typical RT-LAMP assay, displaying the real-time progression of DNA synthesis as measured by fluorescence intensity (RFU) on the y-axis against the elapsed reaction time on the x-axis. The variety of colors corresponds to different template concentrations. The black horizontal line at the lower part of the graph represents no-template controls (NTC), which show no increase in fluorescence, thereby confirming the absence of contamination or non-specific amplification.

Quality control assays

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the product component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the product component in test for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

The Polaris® Lyophilizable RT-LAMP Buffer 6x is tested for performance in in Reverse transcription Loop-mediated isothermal amplification (RT-LAMP) reactions using eukaryotic samples.

Troubleshooting

Troubleshooting RT-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 RT-LAMP amplification using Polaris® Lyophilizable RT-LAMP Buffer 6x. 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 RT-LAMP Buffer 6x 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 RT-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 RT-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/RNases**
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 RT-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 RT-LAMP Buffer 6x 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 RNA. Poor RT-LAMP primer design is responsible for most non-specific amplifications.
• **Cross-contamination**
Create separate work areas for sample processing and RT-LAMP setup. Use separate, dedicated pipettes, and disposable tips for each setup. Always follow good laboratory practices to avoid contamination. Include negative controls (no RNA template) in RT-LAMP reactions to monitor for contamination in reagents or labware.

By systematically addressing these troubleshooting aspects, users can enhance the performance and reliability of RT-LAMP assays conducted with Polaris® Lyophilizable RT-LAMP Buffer 6x. 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.