MD0756_IFU_EN_V2402

Polaris® Speedy LAMP Fluorescent Dye 25x

Catalogue number	Presentation
MD07561	50 µL (50 rxns of 25 µL)
MD07562	200 µL (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 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 LAMP Testing, enabling precise and robust reporting of amplification with enhanced specificity at operational temperatures of 60-72.5°C when combined with Polaris® LAMP enzymes/Master Mixes.
- Specially formulated for high levels of fluorescence, offering superior reporting capacity even of minute amounts DNA.
- Highly developed formula for reaction acceleration, allowing for result reporting in under 5 min.

Description

Polaris® Speedy LAMP Fluorescent Dye 25x is an optimized and highly efficient DNA intercalating fluorescent dye specifically developed for Loop-mediated isothermal amplification (LAMP) assays. The chemistry of this novel LAMP Dye is similar to NZY LAMP Fluorescent dye 50x (MB454) but was specifically designed to accelerate LAMP and RT-LAMP reactions, allowing for even faster time to result values without compromising test accuracy or reliability (Figure 1). Polaris® Speedy LAMP Fluorescent Dye 25x allows for rapid and highly reproducible results on the most common real-time PCR apparatus, given that it is excited and reports fluorescence using the SYBR/FAM channel. Designed for versatility, it allows for the dilution unto a desired reaction concentration, if needed. The latest progresses in LAMP technology were incorporated in the development of this dye, thus allowing for an accurate, reliable, and stable reporting of dsDNA. This guarantees that Polaris® Speedy LAMP Fluorescent Dye 25x delivers unparalleled sensitivity coupled with highly reproducible and ultra-fast, accelerated LAMP protocols. Polaris® Speedy LAMP Fluorescent Dye 25x, through its sophisticated design and extensive validation, emerges as an indispensable asset for the development of LAMP-based diagnostics.

Storage conditions

This product is shipped at room temperature. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. Minimize the number of freeze-thaw cycles by producing working aliquots. 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® Speedy LAMP Fluorescent Dye 25x will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

System Components

This product provides the following set of reagents in sufficient amounts to perform 50 or 200 *in vitro* LAMP/RT-LAMP reactions of 25 µL each.

SKU	COMPONENT		TUBE/BOTTLE	VOLUME
MD07561	**Speedy LAMP dye 25x**	Polaris® Speedy LAMP Fluorescent Dye 25x	1	50 µL
MD07562	**Speedy LAMP dye 25x**	Polaris® Speedy LAMP Fluorescent Dye 25x	1	200 µL

Reagents, Materials and Equipment Required but Not Provided

To ensure the effective use of the Polaris® Speedy LAMP Fluorescent dye 25x, the following reagents and equipment are highly recommended.

- Real-time PCR Instrument: Ensure the instrument can detect 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/RNA contamination, you should assign independent areas for reaction set-up and LAMP/RT-LAMP amplification. It is essential that any tubes containing amplified product should not be opened in the LAMP/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 LAMP/RT-LAMP artifacts, which could compromise the sensitivity and/or specificity of detection.
  - Upon reaction setup, swiftly progress to initiating the LAMP/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 LAMP/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 LAMP/RT-LAMP reactions. While it serves as a reliable starting point, some parameters may require adjustments based on specific needs, such as reagents, 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 components. To avoid cross-contamination, we strongly recommend pipetting the template only after all the other components have been used and remaining material properly stored.

1. Thaw all components on ice. Before any tube is opened, gently 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 LAMP/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)
Polaris® Lyophilizable LAMP/RT-LAMP Master Mix 4x (not provided) (*)	6.25	*n* x 6.25
Primer Mix 10x (not provided)	2.5	*n* x 2.5
Polaris® $MgSO_4$ 100 mM (not provided) (**)	1.5	*n* x 1.5
Polaris® Speedy LAMP Fluorescent Dye 25x	1	*n* x 1
Template (not provided)	Variable	-
DEPC-treated water (not provided) (***)	Variable	-
TOTAL	**25**	***n* x 25**

** We recommend the use of this LAMP/RT-LAMP Master Mix, but Polaris® Speedy LAMP Fluorescent Dye 25x is compatible with the majority of standard master mixes; Concomitantly, levels of $MgSO_4$ may vary for other master mixes used, so please adjust accordingly.*
*** Polaris® LAMP/RT-LAMP Master Mixes include $MgSO_4$ 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.*

3. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the LAMP/RT-LAMP detection steps.
4. Place the reaction plate within the real-time instrument and run the general LAMP/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, 0.5 °C/read	10 second intervals	Melting curve *

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

5. To assess the specificity of the LAMP amplification reaction when performing fluorescence-based real-time LAMP/RT-LAMP, it is highly recommended to include a Melt Curve step: 65 → 99 °C at 10 second intervals.

Important notes

Sample material

Achieving optimal results in LAMP/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 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/ transcriptomic mRNA templates (>100 target copies/reaction).

Primers

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

Testing multiple primer sets with control kits, such as those offered by NZYtech, NZY RT-LAMP Positive Control Kit (Cat. No. MB480), or NZY RT-LAMP Positive Control Kit (Cat. No. MB481), 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/RT-LAMP reaction. A melting 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 T_m 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. When using Polaris® Buffers for LAMP/RT-LAMP, it is essential to note that they already incorporate 2 mM $MgSO_4$ in their formulation.

Negative Control

Including a negative control in LAMP/RT-LAMP assays is crucial to validate positive results and ensure the reliability of the assay. Due to the nature of LAMP/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, 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/RNA template in the reaction mix with molecular-grade ultra-pure water. 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 (MB480) and NZY RT-LAMP Positive Control Kit (Cat. No. MB481), purposedly designed for validating LAMP/RT-LAMP reagents, set-ups, and reactions.

Data

Typical amplification plots obtained with Polaris® Speedy LAMP Fluorescent Dye 25x with samples containing transcriptomic mRNA are illustrated in Figure 1.

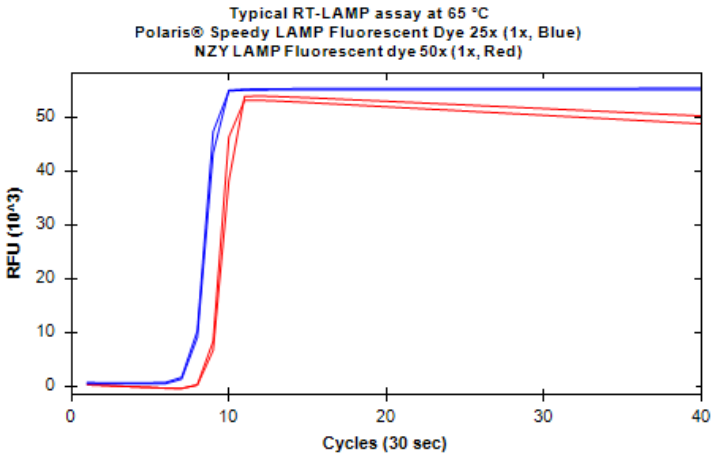

Figure 1. Typical RT-LAMP Amplification Plot. A typical RT-LAMP assay using both Polaris® Speedy LAMP Fluorescent Dye 25x (MD07561/2) (1x, Blue) and NZY LAMP Fluorescent dye 50x (MB454) (1x, Red), at 65 °C.

Quality control assays

Genomic DNA contamination

Polaris® Speedy LAMP Fluorescent Dye 25x is tested to verif that ≤ 0.75 copies of bacterial gDNA and no human DNA are present. This is evaluated through qPCR detection.

Nucleases assay

No nuclease contamination is detected in Polaris® Speedy LAMP Fluorescent Dye 25x 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 plasmid DNA are incubated with each of the Polaris® Speedy LAMP Fluorescent Dye 25x 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 Polaris® Speedy LAMP Fluorescent Dye 25x is tested for performance in Loop-mediated isothermal amplification (LAMP) reactions using eukaryotic samples.

Troubleshooting

Troubleshooting LAMP/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 LAMP/RT-LAMP amplification using Polaris® Speedy LAMP Fluorescent Dye 25x. 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® Speedy LAMP Fluorescent Dye 25x 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. Upon first use, divide into functional aliquots.
• **Excessive amount of sample in the reaction**
You may use up to 50% of the sample/template in a LAMP/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 sample per 25 µL reaction.
• **Suboptimal LAMP/RT-LAMP conditions**
Explore varying the reaction temperature, adjusting reaction time, or 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 – MB47301). Change gloves frequently. If need to store extract samples for an extended period, always freezing them between -65 °C and -85 °C; this will help prevent DNase/RNase activity.
LOW AMPLIFICATION YIELD
• **Ineffective reaction temperature**
Optimize the LAMP/RT-LAMP protocol by tweaking the reaction temperature and time, while observing the impact on amplicon yield.
• **Presence of inhibitors**
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 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/RNA. Poor LAMP/RT-LAMP primer design is responsible for most non-specific amplifications.
• **Cross-contamination**
Create separate work areas for sample processing and LAMP/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 DNA/RNA template) in LAMP/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 LAMP/RT-LAMP assays conducted with Polaris® Speedy LAMP Fluorescent Dye 25x. 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.