NZY LAMP Positive Control Kit

Catalogue number	**Presentation**
MB48001	50x reactions

Description

The NZY LAMP Positive Control Kit is an essential tool for setting up and controlling Loop-mediated isothermal AMPlification (LAMP) assays in various scenarios. This kit allows the production of reliable LAMP reactions and detection of reaction products by means of fluorescence evaluation in real time, similar to a qPCR reaction. Professionals and researchers looking for a reliable and efficient solution for LAMP assay validation will find this kit extremely useful. The provision of a complete set of LAMP reagents allows testing newly developed primer sets with different template types and/or LAMP master mixes by replacing these different components in a well-validated assay. Thus, the ease of use of the assay, coupled with the precision and reliability of the NZY LAMP Positive Control Kit, ensures effective and accurate results in various molecular diagnostic applications. In addition, in educational environments, the kit serves as an effective teaching aid. It provides students and teachers with practical experience in modern molecular diagnostic methods, fostering the development of future scientists by giving them the skills and knowledge they need to innovate in this field. In summary, the NZY LAMP Positive Control Kit is designed to support both discovery and learning in the dynamic fields of molecular biology and molecular diagnostics.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store all components of the kit immediately at -85 °C to -65 °C. These meticulous storage procedures ensure that the NZY LAMP Positive Control Kit delivers consistent and reliable results across its lifespan and usage. All components are formulated ready to use.
The product will remain stable at least up to the expiry date if stored as specified.

Components

The kit provides a comprehensive set of reagents sufficient to perform 50 *in vitro* LAMP reactions of 25 µL each.

COMPONENT	TUBES	VOLUME	CAP COLOR
NZY LAMP Master Mix 4x	1	312.5 µL	Neutral
NZY LAMP Primer Mix 10x	1	125 µL	Neutral
NZY LAMP Positive Control 10^7 copies/µL	1	50 µL	Red
NZY Speedy LAMP Dye 25x	1	50 µL	Brown
100 mM $MgSO_4$	1	500 µL	Neutral
DEPC-treated Water	2	1 mL	Neutral

Reagents, Materials and Equipment Required but Not Provided

To ensure the effective implementation of the NZY LAMP Positive Control Kit, the following reagents and equipment are necessary:

- Real-time fluorimeter (qPCR machine): Equipment capable of producing data in the SYBR/FAM channel is essential for accurate detection and analysis.
- DNA Extraction Kit: When the Kit is used to evaluate newly formulated primer mixes, for optimal results we recommend using DNA templates extracted using NZYtech's DNA extraction kits. These kits are specifically designed and regularly refined to enhance performance in Molecular Diagnostic applications.
- RNase/DNase-Free qPCR Plasticware: This includes PCR tubes, strips, caps, 96-well plates, and adhesive films. NZYtech also provides these high-quality consumables, ensuring compatibility with the Kit.
- Pipettors and Filter Tips: Use RNase/DNase-free pipettors and filter tips to preserve sample integrity and prevent contamination.
- Disposable Gloves: Essential for maintaining a sterile environment and preventing contamination.
- Vortex and Centrifuge: These instruments are essential for achieving efficient reagent homogenization, as well as for sample preparation and processing.

For optimal performance and reliability in your LAMP assays, using equipment and reagents that meet these specifications is crucial. NZYtech's range of products is designed to work seamlessly with the NZY LAMP Positive Control Kit, ensuring high-quality and consistent results.

Rationale for the test

The NZYtech LAMP Positive Control Kit is meticulously designed to generate a robust and highly intense signal in LAMP reactions. Here is how it works:

Isothermal Amplification

Unlike traditional PCR, LAMP operates at a constant temperature, in this case ranging from 68 °C to 70 °C. This isothermal setting facilitates rapid DNA amplification without the need for thermal cycling, making the process faster and energetically more efficient.

Unique Primer Design

This LAMP assay utilizes a set of six specially designed primers that recognize distinct regions on the target DNA sequence. This design ensures high specificity and minimizes the chances of non-specific amplification. Primers initiate the synthesis of DNA strands, leading to the formation of loop structures that serve as templates for further amplification.

Strand Displacement Activity

This LAMP assay employs a DNA polymerase with high strand displacement activity, allowing for continuous synthesis of DNA without the need to remove the existing strands. This feature contributes to the rapid accumulation of DNA products.

Signal Detection

The NZY Speedy LAMP Dye included in the kit 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.

The combination of these molecular mechanisms makes the LAMP assay a powerful tool for the rapid, specific, and sensitive detection of nucleic acids. The NZYtech LAMP Positive Control Kit 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.

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. The kit includes an NZY LAMP Positive Control provided at a concentration of 10^7 copies/µL, which is key to confirming the proper setup of a LAMP reaction. A successful reaction using the positive control should be strong, rapid, and reproducible.

Positive control and Standard Curve Preparation

This kit allows the production of a standard curve of diminishing starting quantities of template. We have verified that this kit will produce a robust, qualifiable amplification result for at least 6 base 10 dilutions of the supplied concentration, i.e., down to 100 copies/µL. Further dilution is possible but might not be as reproducible and predictable as the recommended range. To prepare a different template concentration Standard LAMP curve, proceed as follows:

1. Pipette 90 µL of **DEPC-treated Water** into 5 tubes, labelled 2 to 6.
2. Pipette 10 µL of **NZY LAMP Positive Control** (10^7 copies/µL) into tube 2.
3. Vortex thoroughly and spin.
4. Change tip and pipette 10 µL from tube 2 into tube 3.
5. Vortex thoroughly and spin.
6. Repeat steps 2 (from sequential tubes) to 5 to complete the dilution series. The final template controls will have the following copy number per reaction:

TEMPLATE CONTROL	COPY NUMBER/µL	COPY NUMBER / REACTION (PER 2 µL)
Tube 1 - NZY LAMP Positive Control	10^7	2 x 10^7
Tube 2	10^6	2 x 10^6
Tube 3	10^5	2 x 10^5
Tube 4	10^4	2 x 10^4
Tube 5	10^3	2 x 10^3
Tube 6	100	200

7. Follow the LAMP Standard Protocol as outlined below. Figure 1 illustrates a typical curve resulting from a LAMP assay conducted with the nucleic acid dilutions prepared as per this protocol.

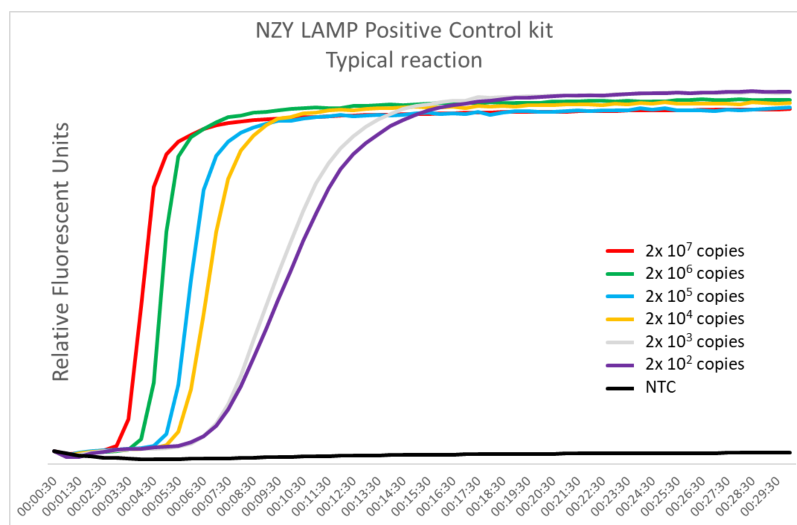

Figure 1: This graph shows the relationship between time (in minutes) and fluorescence for LAMP reactions using this kit and using different template concentrations. The curves demonstrate detection ranging from 2 x 10^7 copies down to 200 copies per reaction, observed through the FAM/SYBR channel. 'NTC' refers to the No Template Control, serving as the negative control.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: Cross-contamination with the positive control template is a critical concern and should be rigorously avoided. To prevent this, handle the template in a separate area from where the reaction mix is prepared. Add the template to the reaction mix away from other components and the negative control, which should be mixed, plated, and sealed before any template handling. If physical separation is not possible, place the negative control as far as possible from the wells containing the template on the plate. Additionally, using different sets of instruments and consumables (e.g., pipettors, tips) for each part of the setup is strongly advised to minimize the risk of cross-contamination. Change gloves regularly.
- Reagents usage: To avoid cross-contamination, we strongly recommend pipetting the NZY LAMP Positive Control template only after having finished handling the other kit components and preparing the mixture detailed in step 2 of the recommended procedure below.
- Controls: Beside the positive control (provided in the kit), we strongly advise including at least one negative control in your experiments. In this control, replace the DNA template with the water provided in the kit. These steps are crucial for ensuring the accuracy and reliability of your results.

Procedure

This standard protocol provides a 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 quantity of the starting template. The effectiveness of this suggested protocol is contingent on the proper storage and condition of all components. For those aiming to evaluate the efficacy of their LAMP assay components, this protocol can be adapted accordingly. Carefully substitute your components into the reaction mix, following this protocol as a template. This approach ensures that any deviations in the assay performance can be attributed to the new components, thereby allowing for an accurate assessment of their effectiveness.

1. Thaw all kit components on ice. Gently vortex all tubes before pulse-spinning them prior to opening any tube.

   Note: avoid using the same vortex and centrifuge used for the template with the other kit components, particularly once they have been used.

2. In the clean reaction setup area, prepare the 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).

COMPONENT	1 REACTION VOLUME (µL)	*n* REACTIONS (*) VOLUME (µL)
NZY LAMP Master Mix 4x	6.25	*n* x 6.25
NZY LAMP Primer Mix 10x	2.5	*n* x 2.5
$MgSO_4$ 100 mM	1.5	*n* x 1.5
NZY Speedy LAMP Fluorescent Dye 25x	1	*n* x 1
DEPC-treated Water	11.75	*n* x 11.75
TOTAL	23	*n* x 23

***** Include sufficient reactions for the No-Template and positive controls. We strongly recommend performing replicates of all reactions.

3. Pipette 23 µL of the LAMP Mix into individual wells, according to your LAMP experimental plate/strip/tube configuration.
4. For the No-Template Control, add 2 µL of DEPC-treated Water, instead of the DNA template, into the no-template control well. The final volume in each well should be 25 µL. Cover wells with appropriate caps.

5. For the Positive Control and eventual respective dilutions, add 2 µL into the positive control wells. The final volume in each well should be 25 µL.
6. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the LAMP detection steps.
7. Place the reaction plate within the real-time instrument and run the general 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 (68-70 °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.*

Technical Notes

Sample Material

When testing the efficacy of newly designed primer mixes, ensure the usage of purified DNA templates for amplification. NZY LAMP Positive Control Kit is compatible with all nucleic acid samples that are suitable for PCR amplification. The quality of nucleic acids can be significantly influenced by the procedures used for collecting biological samples, as well as the conditions during shipping, storage, and processing. Therefore, it is important to optimize these procedures to ensure high-quality nucleic acid samples. To verify the suitability of samples for LAMP amplification we recommend evaluating DNA integrity and purity through agarose gel electrophoresis and/or spectrophotometric analysis. For further purity analysis, a preliminary LAMP assay should also be performed, where your sample is tested in a standard reaction using all of the kit's components. In parallel, a control reaction should also be prepared where your sample is absent. If the nucleic acids in your sample are sufficiently pure, there should be no significant difference in the amplification profile between the two reactions.

Quality control

Purity

All enzymes present in this kit were subjected to purity assessment tests, where > 90% minimum purity was judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

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 kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit 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

NZY LAMP Positive Control Kit is tested for performance in Loop-mediated isothermal amplification (LAMP) reactions using human and viral samples.

Troubleshooting

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. A major usefulness of this kit is that it can be used to verify the quality or integrity of other LAMP reagents one might desire, by replacing the ones supplied with their own. However, we want to emphasize that these alterations are not guaranteed to allow for successful reactions and, as such, the following troubleshooting suggestions are merely speculative and are based on our own experience. If you are having issues with a particular reagent that is not compatible with this Kit, we are willing to try to help you achieve the best possible results. 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 NZY LAMP Positive Control Kit 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 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 reaction time, or increasing the amplification protocol over 30 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 contamination. Use DNase-free, autoclaved, or sterile equipment whenever possible. Use DNase-free water (we highly recommend using the water provided in the Kit). Change gloves frequently. If need to store extract samples for an extended period, consider freezing them at -20 °C or even at -80 °C; this can help prevent DNase 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, NZY LAMP Master Mix is 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 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 genomic 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.

For life science research only. Not for use in diagnostic procedures.

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