NZYtech's LAMP and RT-LAMP Positive Control kits: invaluable tools in isothermal test development and use

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Introduction

Isothermal amplification techniques such as loop-mediated isothermal amplification (LAMP) have gathered recent attention due to their immediate advantages over traditional qPCR testing. LAMP assays do not require thermal cycling, reducing equipment needs and improving time to result values due to the technique's intricate steps and reaction sequences, as well as the elevated processivity of its critical enzyme, Bst DNA polymerase[1, 2].

Although LAMP assays offer high target specificity, the complex reaction matrix which involves up to six primer sequences and a relatively low reaction temperature (55-65 ºC) often leads to non-specific amplifications[3]. Adding to this, primer design requirements are less straightforward than a typical PCR reaction, while probe insertion is a challenging design task due to the lack of 5'→3' exonuclease activity by Bst[4,5]. These hurdles are manageable in well-established LAMP and RT-LAMP protocols, but in earlier phases of development, in troubleshooting established protocols, where certitude of result is arguably the most critical, these might render progress prohibitively slow and costly.

It is with these issues in mind that NZYtech has developed LAMP and RT-LAMP Positive Control Kits, two novel products that contain all the individualized components required to produce a fully functional LAMP/ RT-LAMP reaction, or to mix and match different reaction components to assess their usefulness. In this matter, these kits allow for a quick, cost-efficient and reliable means to test new reagents, primers, enzymes or any other reaction elements one might want to test. They are also a great tool for any researcher starting out in isothermal amplification procedures, as well as powerful teaching aides, allowing for customizable and hands-on learning of the role and importance of the various elements of a LAMP/ RT-LAMP reaction.

In this application note, we detail and characterize the use of NZYtech's LAMP and RT-LAMP Positive Control kits, with the suggestion of a reaction protocol and tips for overcoming any issues one might have in isothermal amplification.

References

1. Notomi, T. *et al.* (2000). Loop-mediated isothermal amplification of DNA.; *Nucleic Acids Research*, 28(12):E63.

2. Parida, M. *et al.* (2008). Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases.; *Reviews in Medical Virology*, 18: 407-421.

3. Wang, D. G. *et al.* (2015). Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification; *Molecules*, 20(4), 6048-6059.

4. Aliotta, J. M. *et al.* (1996). Thermostable *Bst* DNA Polymerase I lacks a 3'→5' proofreading exonuclease activity; *Genetic Analysis: Biomolecular Engineering*, 12(5-6), 185-195.

5. Ye, X. *et al.* (2018). A novel exonuclease-assisted isothermal nucleic acid amplification with ultrahigh specificity mediated by full-length Bst DNA polymerase; *Chemical Communications*, 54(75), 10562-10565.

Materials & Methods

- LAMP/ RT-LAMP Positive Control kit (MB0480/MB0481).
- (*Optional*): Any other reaction element that one might want to test/replace the one provided with.

This standard protocol provides a foundational guideline for conducting LAMP/ RT-LAMP reactions and can be optimized according to the instructions provided in the "Guidelines" section of this application note:

1. The reaction mix was set up in a sterile environment following the instructions outlined in Table 1, using nuclease-free materials and adding water up to the final reaction volume. The total reaction volume prepared was defined depending on the number of replicates established for each case. In the example below, a base 10 dilution of the supplied template was performed, down to 100 copies/ µL. This allowed the performing of a serial dilution experiment with 6 dilution points.

Table 1. Reaction setup.

Component	Volume
NZY LAMP/ RT-LAMP Master Mix 4x	6.25 µL
$MgSO_4$ 100 mM	1.5 µL
NZY LAMP Primer Mix 10x	2.5 µL
NZY Speedy LAMP Dye 25x	1 µL
NZY LAMP Positive Control 10^7 -10^2 copies/µL	2 µL
DEPC-treated Water	11.75 µL
TOTAL	25 µL

Note: *Any element may be replaced by an alternative component if necessary, or added in, reducing the equivalent volume of DEPC-treated water up to 1175 µL of novel material in the reaction.*

2. Real-time LAMP/ RT-LAMP were performed on a Bio-Rad™ CFX Opus™ Real-Time PCR System or in an Applied Biosystems™ StepOnePlus™ using the protocol detailed in Table 2:

Table 2. Cycling protocol.

Number of cycles	Step	Temperature	Time
60	Amplification	69 ºC	30 sec
1	Inactivation	95 ºC	3 min
-	Melting curve	70 – 99 ºC	0.5 ºC increment at 10 second intervals

Note: *Time can be extended, and temperature can be adjusted (between 65 ˚C to 70 ˚C) as necessary whenever amplification times have been previously reported as extensive.*

3. Bio-Rad™ CFX Maestro™ Real-Time PCR Software or Applied Biosystems™ StepOnePlus™ Software were used for data analysis.

Guidelines

- Ensure homogeneity of the reagents before use. To achieve this, gently flick the tubes provided and centrifuge for a few seconds to collect the volume at the bottom of the tube. Maintain tubes on ice.

- To help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up and LAMP/ RT-LAMP amplification.

- Mg^{2+} concentration optimization can be performed by testing a range between 2 – 16 mM of $MgSO_4$ final concentration. Please bear in mind that the supplied LAMP/ RT-LAMP Master Mixes already contain 2 mM of $MgSO_4$ concentration.

- Including a negative control in LAMP/ RT-LAMP assays 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 LAMP /RT-LAMP assay, simply replace the volume of template with DEPC-treated water.

Results

Fast, robust, and reliable LAMP/ RT-LAMP amplification

The amplification capability of NZY LAMP/ RT-LAMP Positive Control kits was evaluated in a real-time setup as detailed in the previous section. A serial dilution of both templates was performed down to a 1:1 000 000 dilution, i.e., down to 1x10^2 copies/µL. Figure 1 details a typical reaction using these kits.

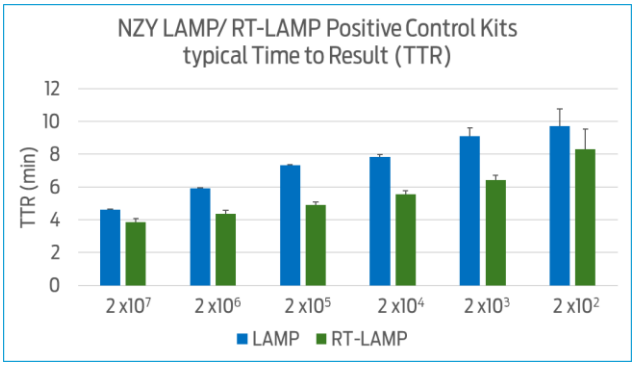

Figure 1. Evaluation of NZY LAMP/ RT-LAMP Positive Control Kits performance (expressed in time to result in minutes). Bars represent Mean + Standard Deviation (n=4) of real-time LAMP/ RT-LAMP assays used to amplify 6 dilutions + NTC (not shown) of the supplied templates, as detailed in the Materials and Methods section. Reactions took place at 69 °C.

The results show that NZY LAMP/ RT-LAMP Positive Control kits were able to quickly (under 10 min), effectively (100% amplification) and accurately (under 10% data spread in all data sets) amplify the supplied templates, even when dilute 1: 1000 000 (Figure 1), confirming a robust performance. The data demonstrate the kits' value for the validation, testing, or optimization of isothermal reactions.

The graphical representation of the amplification curves, shown in Figures 2 and 3, reveals distinguishable amplification curves for each dilution. Furthermore, virtually no non-specific amplification was seen on NTC samples and, even when present, these were clearly distinguishable by Melting Curve analysis (data not shown).

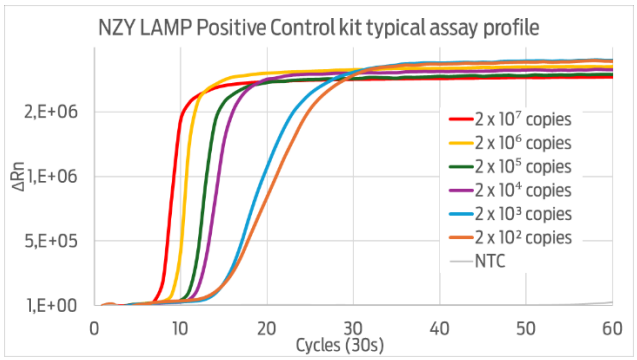

Figure 2. Typical amplification plots using the NZY LAMP Positive Control Kit with various starting template DNA quantities: 2 x 10^7 copies (—), 2 x 10^6 copies (—), 2 x 10^5 copies (—), 2 x 10^4 copies (—), 2 x 10^3 copies (—), 2 x 10^2 copies (—) and No Template Control (—). Each cycle has a duration of 30 seconds.

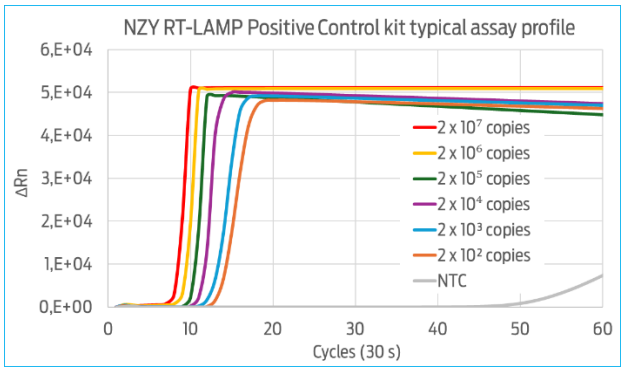

Figure 3. Typical amplification plots using NZY RT-LAMP Positive Control Kit with various starting template RNA concentrations: 2 x 10^7 copies (—), 2 x 10^6 copies (—), 2 x 10^5 copies (—), 2 x 10^4 copies (—), 2 x 10^3 copies (—), 2 x 10^2 copies (—) and No Template Control (—). Each cycle has a duration of 30 seconds.

Conclusions

The data presented here reveal that the NZY LAMP/ RT-LAMP Positive Control kits are excellent tools for assay development and optimization in the context of isothermal amplification. Potential applications of these Kits include LAMP tools validation, development of reaction variations, exploration of additive or inhibitor effects, or simply experimenting with LAMP/ RT-LAMP technology, for instance in a classroom setting. These kits exhibit consistent reproducibility, short time-to-result, and reliability. The proprietary Bst enzyme allows for unparalleled processivity at higher than usual temperatures, allowing for fast, accurate and controlled NTC amplification reactions.

Furthermore, NZY LAMP/ RT-LAMP Positive Control kits are sensitive enough to clearly and unequivocally amplify at least 200 copies of the supplied DNA/RNA templates, a testimony to not only the enzymes and accompanying Master Mixes, but also to the selected primer sets and supplied templates, that serve as a benchmark of reproducibility, robustness and reliability for any developing LAMP/ RT-LAMP assays.

In summary, NZY LAMP/ RT-LAMP Positive Control kits are powerful tools for discovery, validation and investigation on isothermal loop-mediated amplification, providing an essential resource for diverse molecular research and diagnostics needs.

Ordering Information

Catalogue number	Product name	Number of reactions
MB48001	NZY LAMP Positive Control Kit	50 rxns
MB48101	NZY RT-LAMP Positive Control Kit	50 rxns

Related products

BstY Polymerases

Catalogue number	Product name	Number of reactions
MD06771	Polaris® Glycerol-free BstY Polymerase 80 U/µL	1 mL (10k rxns of 25 µL)
MD06772	Polaris® Glycerol-free BstY Polymerase 80 U/µL	5 x 20 µL (1k rxns of 25 µL)
MD06781	Polaris® BstY Polymerase 8 U/µL	5 mL (5k rxns of 25 µL)
MD06782	Polaris® BstY Polymerase 8 U/µL	5 x 200 µL (1k rxns of 25 µL)
MD06791	Polaris® Lyo BstY Polymerase 40 kU	for 25 x 200 µL (5k rxns of 25 µL)
MD06792	Polaris® Lyo BstY Polymerase 40 kU	for 5 x 200 µL (1k rxns of 25 µL)

Buffers

Catalogue number	Product name	Number of reactions
MD06881	Polaris® BstY Reaction Buffer 10x	12.5 mL
MD06882	Polaris® BstY Reaction Buffer 10x	2 x 12.5 mL
MD06841	Polaris® Lyophilizable LAMP Buffer 6x	21 mL
MD06842	Polaris® Lyophilizable LAMP Buffer 6x	2 x 21 mL

(RT)-qPCR Enzymes & Additives

Catalogue number	Product name	Number of reactions
MD07711	Polaris® Lyophilizable LAMP Pack	200 rxns of 25 µL
MD07721	Polaris® LAMP Pack	200 rxns of 25 µL
MD07731	Polaris® Lyo LAMP Pack	200 rxns of 25 µL
MD06801	Polaris® Lyophilizable LAMP Master Mix 4x	312.5 µL (50 rxns of 25 µL)
MD06802	Polaris® Lyophilizable LAMP Master Mix 4x	25 mL (4k rxns of 25 µL)
MD06961	Polaris® Lyo LAMP Master Mix 4x	for 660 uL (100 rxns)
MD06962	Polaris® Lyo LAMP Master Mix 4x	for 25 x 660 uL (2500 rxns)
MD06811	Polaris® Lyophilizable RT-LAMP Master Mix 4x	312.5 µL (50 rxns of 25 µL)
MD06812	Polaris® Lyophilizable RT-LAMP Master Mix 4x	25 mL (4k rxns of 25 µL)
MD06971	Polaris® Lyo RT-LAMP Master Mix 4x	for 660 uL (100 rxns)
MD06972	Polaris® Lyo RT-LAMP Master Mix 4x	for 25 x 660 uL (2500 rxns)

Catalogue number	Product name	Number of reactions
MD06891	Polaris® $MgSO_4$ 100 mM	12.5 mL
MD06892	Polaris® $MgSO_4$ 100 mM	2 x 12.5 mL
MD07561	Polaris® Speedy LAMP Fluorescent Dye 25x	50 µL (50 rxns of 25 µL)
MD07562	Polaris® Speedy LAMP Fluorescent Dye 25x	200 µL (200 rxns of 25 µL)