NZY LAMP Fluorescent dye 50x

Catalogue number:

MB45401, 50x (250 µL)

Description

NZY LAMP Fluorescent dye 50x is an optimized and highly efficient DNA intercalating fluorescent dye directly developed for Loop-mediated isothermal AMPlification (LAMP) assays. This fluorescent dye enables fast and highly reproducible procedures on the most common real-time PCR apparatus, given that it is excited and reports fluorescence using the SYBR/FAM channel, a ubiquitous fluorescence channel. The latest progresses in LAMP technology were incorporated in the development of this dye, thus allowing for an accurate, reliable, and stable reporting of DNA. This guarantees that NZY LAMP Fluorescent dye 50x delivers sensitivity coupled with highly reproducible and fast LAMP protocols.

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

This molecular reagent should be stored at -85 to -15°C in a freezer. Minimize the number of freeze-thaw cycles by producing working aliquots. The product will remain stable till the expiry date if stored as specified. Keep protected from the light.

Compatible real-time PCR instruments

This fluorescent dye is compatible with all real-time PCR apparatus that possess the SYBR/FAM channel.

It has been validated in instruments from various suppliers, such as Bio-Rad (CFX96 Touch and CFX96 Opus), Applied Biosystems (StepOne Plus, 7500 Fast, QuantiStudio 5), amongst others.

Protocol

The following protocol serves as a general guideline and a starting point for any qLAMP procedure. Optimal reaction conditions (incubation times and temperatures or concentration of DNA template) may vary and, in particular conditions, may require further optimization.

Reaction mix composition: the given volumes are based on a standard 25 µL final reaction mix which can be scale adjusted.

Polaris™ LAMP Master Mix 4x, IVD (not provided)	6.25 µL	1×
10x primer mix (not provided)	2.5 µL	Variable (*)
100 mM $MgSO_4$ (not provided)	1.5 µL	6 mM
NZY LAMP Fluorescent dye 50x	0.5 µL	1x
Template (not provided)	Variable	100 to 10^6 copies are recommended
Nuclease-free water (not provided)	Up to 25 µL	

(¥) See section of "General considerations" below for more details about primers design and final concentrations in the reaction.

Suggested amplification protocol

NZY LAMP Fluorescent dye 50x was optimized for the detection of DNA in a LAMP setting. The table below displays a standard LAMP experimental protocol optimized on several platforms. However, these conditions may be adapted to suit different machine-specific protocols.

Cycles	Temp.	Time	Notes
60	66 °C	30 s	Polymerase activity
1	95 °C	120 s	Enzyme inactivation
1	60-100 °C, 0.5 °C/ read	5 s intervals	Melting curve

General considerations

To prevent any DNA contamination, we recommend that users have independent areas for reaction set-up, template incorporation, LAMP amplification and post-LAMP gel analysis in case this is required. It is essential that any tubes containing amplified LAMP products are not opened in the LAMP set-up area.

Primers: These guidelines refer to the design and set up of LAMP DNA amplification. In its simplest version, a LAMP assay typically includes a set of 4 primers (2 outer primers and 2 inner primers) that recognize distinct regions of the target gene. These are commonly referred to as FIP/BIP (Forward/Backward Inner Primer) and F3/B3 (Forward/Backward Outer Primer). The addition of two further loop primers (FL/BL – Forward/Backward Loop Primer) significantly accelerates reaction time, resulting in reduced Ct values.

The specific amplification, yield and overall efficiency of any LAMP reaction can be critically affected by the sequence and concentration of the primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your LAMP experiment:

- Since there are 2-3 primer sequences in the mix, a highly detailed design is required to minimize the formation of primer dimers. In general, it is highly recommended that a few sets of primers are designed for the same target and tested before validation (there are a few online LAMP primer design tools that tackle these issues and should be used).
- Distance between FIP and BIP primers should be between 120 and 160 bp.
- GC content should be between 45-60%; avoid single or dinucleotide base repeats and probable secondary structure regions.
- Finally, the amplicon comprised should be below 300 bp.

It is also highly recommended that a 10x primer mix is made and used to feed the reaction mix. We recommend that this mix should be made from 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4-8 µM FL and 4-8 µM BL.

Template: The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend the use of 10-500 ng of cDNA/ genomic DNA template.

Quality control assays

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through PCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the NZY LAMP Fluorescent dye for 14-16 hours at 37 °C. RNase contamination is screened against by incubating 1 µg of RNA with NZY LAMP Fluorescent dye for 1h at 37 °C. Following incubation, the nucleic acid is visualised on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

NZY LAMP Fluorescent dye 50x is extensively tested for reporting stability.

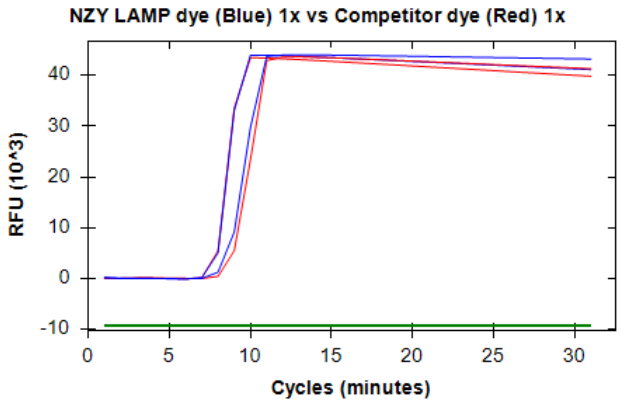

Figure above reports a typical qLAMP assay using both NZY LAMP Fluorescent dye 50x (1x, Blue) and a competitor dye (1x, Red), at 66 °C.

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Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.:+351.213643514 Fax: +351.217151168
www.nzytech.com