Flo-LAMP UNG KIT

LAMP (Loop-mediated isothermal amplification) is a technique for a quick amplification of nucleic acids. In contrast to the PCR technique, in which the reaction is carried out with a series of alternating temperature cycles, the LAMP reaction is carried out in a constant temperature of 65°C. This technique uses Opti Bst polymerase which has strand displacement activity. The reaction is exceptionally quick and efficient thanks to using a set of three pairs of primers specific for template DNA. In the first phase, a base product with loop formation at the ends is created, next the strand displacement activity and attachment of subsequent primers lead to the creation of increasingly longer DNA fragments consisting of the base product replications. In the electrophoretic separation the obtained product is not a single band but constitutes a mixture of products of various sizes with repeated sequence. The detection is done by fluorescence, which allows for the measurement in real time. During the reaction, an insoluble precipitate of magnesium pyrophosphate is formed, which can be observed as turbidity. Flo-LAMP UNG KIT contains dUTP and Thermolabile UNG, which prevents amplification of previous reaction products that contain uracil. These additions reduce the possibility of carryover contamination (where the product of a previous reaction can unexpectedly serve as the substrate of a subsequent reaction).

Kit includes:

Component	**Cat. No. E1421-01** **100 reactions, 25 µl each**	**Cat. No. E1421-02** **500 reactions, 25 µl each**
Flo-LAMP UNG Reaction Mix (2x) orange cap	1300 µl	5 x 1300 µl
Opti Bst DNA polymerase with UNG yellow cap	110 µl	5 x 110 µl
Flo – fluorescent dye (FAM) brown tube	110 µl	5 x 110 µl
Lambda Positive Control (10x) (Lambda DNA and primers mix) black cap	70 µl	350 µl
RNase free Water transparent cap	1200 µl	5 x 1200 µl

All kit components should be stored at a temperature of -20°C

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Protocol:
Designing of primers:

LAMP reaction to a large extent depends on the sequence of primers so it is recommended to test several sets of primers to optimise the process. For primers designing we recommend *PrimerExplorer* software available online.

Dilution of primers:

Prepare 10x Primer Mix of six primers diluted with RNase free water according to the table below. The prepared 10x Primer Mix may be stored at a temperature of –20°C until its next use.

Note: Primers desalted by precipitation are of sufficient purity to perform the reaction. Primers do not need to be purified by HPLC. Diluting of primers should be carried out in the place where work with template is not performed.

Primers	FIP 100 µM	BIP 100 µM	F3 100 µM	B3 100 µM	LF 100 µM	LB 100 µM	RNase free water
10x Primer Mix concentration	16 µM	16 µM	2 µM	2 µM	4 µM	4 µM	
Volume 1000 µl	160 µl	160 µl	20 µl	20 µl	40 µl	40 µl	560 µl

Dilution of the template :

The DNA template should be diluted in RNase free water or in TE buffer. It should be done outside the room used for the preparation or detection of the reaction. The typical template concentration is within the range 500 – 0.05 ng/reaction.

Preparation of the reaction:

The LAMP reaction should be prepared on ice or in a cooling block according to the table below. The reaction should be mixed by pipetting, and immediately placed in a cycler previously warmed up to 65°C. It is always recommended to prepare negative control (N).

Reaction component Master Mix	**Specific reaction C**	**Negative control N**	**Positive control P Lambda DNA**
Flo-LAMP UNG Reaction Mix (2x)	12,5 µl	12,5 µl	12,5 µl
10x Primer Mix	2,5 µl	2,5 µl	-
Opti Bst DNA polymerase with UNG	1 µl	1 µl	1 µl
Flo – fluorescent dye (FAM)	1 µl	1 µl	1 µl
Lambda Positive Control (10x)	-	-	2,5 µl
Template	variable	-	-
RNase free Water	variable	8 µl	8 µl
Reaction volume	25 µl	25 µl	25 µl

For the detection with the fluorescent dye, it is recommended to set 40 one-minute cycles with measurement in Channel 1 (FAM) or (SYBR) according to the table below. If the number of template copies is less than 200, it is recommended to extend the reaction time to 50 minutes (prelonged incubation may cause amplification of unspecifically annealed primers or primer dimer formations).

Reaction	Temperature	Duration	Number of cycles
Flo-LAMP UNG reaction mix	65-68°C	1 min**	30-50*
	85°C	5 min	1

* number of cycles depends on the reaction optimisation
** fluorescence reading after every minute

Additional information:

1. For difficult DNA templates pre-warm the reaction mix **witout enzyme** at 92°C for 1 min. Cool down to room temperature, then add the Opti Bst DNA Polymerase, mix well, centrifuge and place tubes into a pre-heated thermocycler or heat block.
2. If amplification in no-template controls occurs the following points should be reviewed.
   - Replace reagent stocks and pre-mixes with new components.
   - Use separate preparation area and equipment if post-reaction processing is necessary.
   - Avoid opening reaction tubes after amplification.
   - Melt curve analysis. Non-template amplification products can be discriminated from positive reactions using differences in their respective melt curves.

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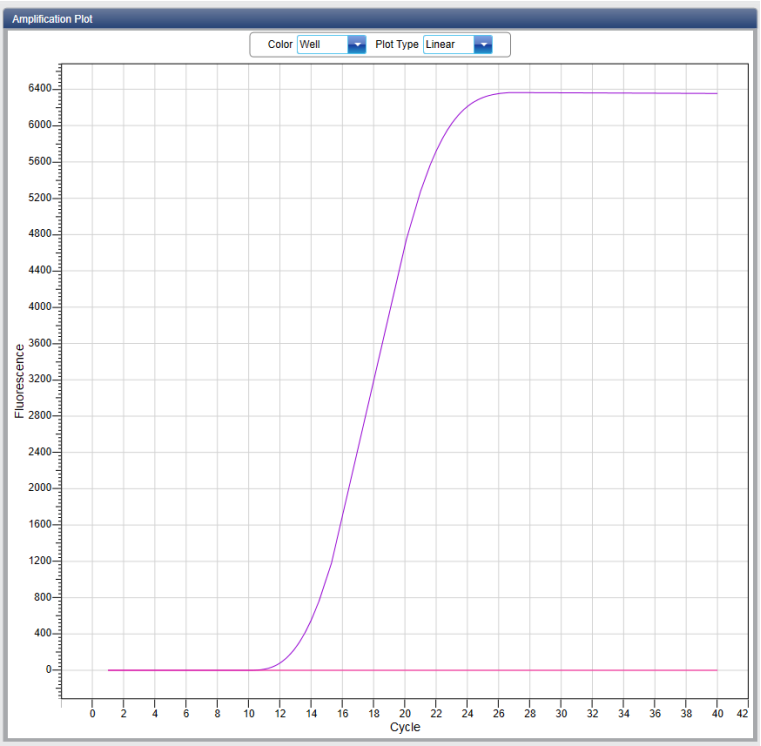
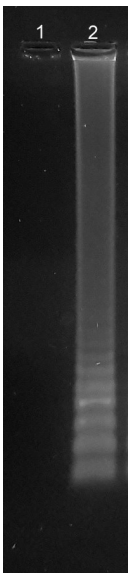
Analysis of results:



Real-time reading with the use of the fluorescence dye.
One-minute cycle, total of 40 cycles, FAM channel: violet — positive control (P); pink — negative control (N).



Agarose gel electrophoretic separation.
1% agarose in 1xTBE: 1 – negative control (N), 2— positive control (P)

Due to substantial amounts of DNA created as a result of the LAMP reaction, it is advised against opening the samples for detection (e.g. electrophoretic separation) after the reaction. There is high risk of contaminating subsequent reactions which may lead to unreliable results.

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