



Limited liability company

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## BioMaster LAMP (2×)

Cat. number MH050-400, MH050-2040

### Description:

BioMaster LAMP SYBR (2×) kit contains 2× reaction mixture BioMaster LAMP SYBR (2×) and sterilized water. 2× reaction mixture BioMaster LAMP SYBR (2×) was designed to perform loop isothermal amplification (LAMP) in real time, using SYBR Green I fluorescent dye. BioMaster LAMP SYBR (2×) contains all the essential reaction components (excluding DNA-matrix and primers):

- high-processive recombinant DNA-polymerase large fragment (*LF*) *Bst*
- deoxynucleoside triphosphate mix
- buffer
- Mg<sup>2+</sup> (6 mM)
- SYBR Green I
- Inert dye.

The mixture was optimized to perform effective and replicable LAMP in real time with genomic, plasmid and viral DNA samples. The mixture contains additives, increasing *Bst* *LF* DNA polymerase half-life and processivity by stability increment during the reaction.

Presented PCR kit composition saves time and decreases contamination possibility due to the small number of pipetting steps. Inert dye in BioMaster LAMP SYBR (2×) stains it blue, facilitating the mixture distribution while using a multiwell microplates.

### Kit contains:

Catalogue number	BioMaster LAMP SYBR (2×)	Water	Amount of 25 µl reactions
MH050-400	4 × 1.25 ml	4 × 1.25 ml	400
MH050-2040	17 × 1.5 ml	3 × 1.8 ml	2040

### BioMaster LAMP SYBR (2×) ingredients:

100 mM Tris-HCl, pH 8.9, 20 mM KCl, 2 mM of each deoxynucleoside triphosphate, 12 mM MgCl<sub>2</sub>, 0.06 activity U/µl *Bst LF* DNA-polymerase, 0,5% Tween 20, *Bst* DNA-polymerase stabilizers, SYBR Green I, inert dye.

### Application:

- loop isothermal amplification (LAMP) in real time, using SYBR Green I fluorescent dye;
- loop isothermal amplification (LAMP) with end-point detection.

### Polymerase properties

DNA-polymerase *LF Bst* is a large *Bst* (*Bacillus stearothermophilus*) polymerase fragment (67 kDa polypeptide), extracted from *E.coli* strain, carrying modified cloned gene. Fragment has a 5'→ 3' -polymerase activity, but lacks 5'→ 3' and 3'→ 5'-exonuclease activity, that allows the application for the isothermal amplification

performance, including LAMP (Loop-Mediated Isothermal Amplification). DNA-polymerase LF *Bst* DNA-polymerase has high DNA-chain displacing activity and can be used for isothermal DNA amplification. The enzyme has the highest activity at 60–65° C.

### **SYBR Green I**

**SYBR Green I** - fluorescent intercalating dye for quantitative and qualitative detection of PCR-products throughout real-time PCR. During the amplification dye SYBR Green I is integrated into the small DNA groove of PCR products and emits a stronger fluorescence signal in comparison with the unbound dye. SYBR Green I absorption and emission maximums are 494 nm and 521 nm, respectively, which allows its application with all currently used real-time PCR instruments.

### **Inert dye**

Inert dye in *BioMaster LAMP SYBR (2x)* does not decrease PCE efficiency and helps to control the mixture distribution process, using multiwell microplates. Adsorption maxima corresponds to 615 nm.

### **Application advantages**

- The mixture is dyed to facilitate the distribution;
- Reaction preparation time decrement;
- The possibility of contamination during PCR components mixing reduction;
- Condition of same type reaction setting is standardized (mixing PCR components across experiments is reduced).

### **Amplification protocol**

1. Thaw the reaction mixture and mix thoroughly. Ice or cooled thermostated rack for reaction performance.
2. Add the next components, estimated for single 25 µl reaction mixture volume, in thin-wall test tubes:

Component	Volume	Final concentration
BioMaster LAMP SYBR (2x)	12,5	1x
Primer mixture	variable	1– 2 µM
DNA-matrix	variable	100 pg – 1 µg
Sterilized water	up to 25 µ	

3. Mix carefully and discard the droplets, using centrifuge.
4. Carry out the reaction at 65 °C. For real-time detection the appropriate amplification program being: 65 °C – 50 sec and plate reading each of 30–40 cycles.

**Storage:** at -20°C, protected from direct light for 18 months; with max. of 50 freeze-thaw cycles.

**Transportation:** in thermostated containers with cooling elements, tolerating temperature increment up to environment temperature, if transported in 10 days.