BIOLABMIX LLC
Taxpayer Identification Number (INN) 5408278957
Tax Registration Reason Code (KPP) 540801001
Ingenernaya st., 28
630090, Novosibirsk,
Russian Federation
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

BioMaster RT-LAMP SYBR (2×)

Cat. No. RM07-80, RM07-400

Description

The kit was designed to perform reverse transcription (RT) and isothermal amplification (LAMP) in a single tube in real-time, using fluorescent SYBR Green I dye. **BioMaster RT-LAMP SYBR (2×)** kit contains **2× RT-LAMP buffer with SYBR, 25× Biomaster RT-LAMP-mix** and **DEPC treated water**. **2× *RT-LAMP* buffer with SYBR** ingredients include all the necessary reaction components (excluding enzymes, DNA-matrix, and primers): buffer component; nucleoside triphosphate mix; Mg^{2+} (6 мМ) ions; intercalating dye SYBR Green I; inert dye.

25× BioMaster RT-LAMP-микс contains revertase RNAscribe RT and LF Bst DNA-polymerase in optimal ratio in order for both reactions to take place.

RNAscribe RT – genetically modified reverse transcriptase (revertase) of murine leukemia virus (*M-MuLV*). Enzyme shows RNA- and DNA-dependent polymerase activity and has optimal conditions at 55 °C (remaining active up to 65 °C). The enzyme has an ability to synthise the first cDNA chain with the length up to 9 t.p., and also to include modified bases. Its high reaction velocity allows to perform the synthesis in 15 minutes only, and high working temperature (up to 65 °C) grants large reaction yield and high reaction specificity.

LF *Bst* DNA-polymerase is a large fragment of *Bst* (*Bacillus stearothermophilus*) polymerase (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.

2× RT-LAMP buffer with SYBR has been optimized for both RT or LAMP effective performance in real-time. Additives and enhancers in it allow to conduct effective RT-LAMP with complicated and GC-rich matrixes. 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 contents

Cat.No.	2× *RT-LAMP* buffer with SYBR	25× BioMaster RT-LAMP-mix	DEPC treated water	Amount of 25 µl reactions
RM07-80	2 × 0.5 ml	1 × 80 µl	2 × 0.5 ml	80
RM07-400	4 × 1.25 ml	1 × 400 µl	3 × 1.8 ml	400

BioMaster RT-LAMP SYBR (2×) ingredients:
100 mM Tris-HCl, pH 8.9, 20 mM KCl, 2 mM of each nucleoside triphosphate, 12 mM $MgCl_2$, 0.06 UA/µl *Bst LF* DNA-polymerase, 0,5% Tween 20, stabilizers of *Bst LF* DNA-polymerase, SYBR Green I, inert dye.

BioMaster RT-LAMP-mix ingredients:
50 mM Tris-HCl, pH 8.0 (at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, 50 % (v/v) glycerin and 0.1 % (v/v) NP-40, RNAse inhibitor, *RNAscribe RT* revertase and LF *Bst*DNA-polymerase.

Application area:

- one-step reverse transcription (RT) and loop isothermal amplification (LAMP) in real-time, using intercalating SYBR Green I dye
- real-time loop isothermal amplification, using intercalating SYBR Green I dye
- real-time loop isothermal amplification with end-point detection

SYBR Green I
SYBR Green I - fluorescent intercalating dye for quantitive and qualitive 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 2× *RT-LAMP* buffer with SYBR does not decrease PCE efficiency and helps to control the mixture distribution process, using multiwell microplates. Adsorption maxima corresponds to 615 nm.

Application advantages

- High sensitivity (10 pg – 1 µg RNA);
- 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. 2. Add the next components, estimated for single 25 µl reaction mixture volume, in thin-wall test tubes:

Component	Volume	Final concentration
2× RT-LAMP buffer with SYBR	12,5	1×
25× BioMaster RT-LAMP-mix	1	1×
Primer mix	variable	1– 2 µM
RNA-matrix	variable	100 pg – 1 µg
DEPC treated water	up to 25 µl	

3. Mix carefully and discard the droplets, using centrifuge.

4. Carry out the reaction at 65 °C. For real-time detection the appropriate amplificatory with the 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 30 freeze-thaw cycles.

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