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BioMaster RT-LAMP (2x)

Cat. No. RM08-80, RM08-400

Description

The kit was designed to perform reverse transcription (RT) and isothermal amplification (LAMP) in a single tube. **BioMaster RT-LAMP (2x)** kit contains **2x RT-LAMP buffer**, **25x Biomaster RT-LAMP-mix** and **DEPC treated water**. **2x RT-LAMP buffer** ingredients include all the necessary reaction components (excluding enzymes, DNA-matrix, and primers): buffer component; deoxynucleoside triphosphate mix; Mg²⁺ (6 mM) ions; inert dye.

25x 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 synthesise 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.

2x RT-LAMP buffer 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. **6x gel application buffer** facilitating sample preparation and control of gel electrophoresis.

Kit contents

Cat.No.	2× RT-LAMP buffer	25× BioMaster RT-LAMP-mix	DEPC treated water	6× application buffer	Amount of 25 µl reactions
RM08-80	2 × 0.5 ml	1 × 80 µl	2 × 0.5 ml	1 × 0.5 ml	80
RM08-400	4 × 1.25 ml	1 × 400 µl	3 × 1.8 ml	1 × 1 ml	400

BioMaster RT-LAMP (2×) ingredients:

100 mM Tris-HCl, pH 8.9, 20 mM KCl, 2 mM each nucleoside triphosphate, 12 mM MgCl₂, 0.06 UA/µl *Bst* LFDNA-polymerase, 0.5% Tween 20, stabilizers.

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
- real-time loop isothermal amplification with end-point detection

Application advantages

- High sensitivity (10 pg – 1 µg RNA);
- Reaction preparation time decrement;
- The possibility of contamination during PCR components mixing reduction.

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
2× RT-LAMP buffer	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.