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BioMaster RT-LAMP-Color (2×)

Cat. number RM06-40, RM06-200

Description:

The kit designed to perform ***colorimetric*** reverse transcription (RT) and loop-mediated isothermal amplification (LAMP) in one tube. ***BioMaster RT-LAMP-Color (2×)*** contains **2× RT-LAMP-Color buffer**; **25× BioMaster *RT-LAMP*-mix,** and **DEPC treated water**. **2× RT-LAMP-Color buffer** includes all of the necessary reaction components (excluding enzymes, DNA-matrix, and primers): low-capacity buffer component; deoxynucleosidetriphosphate mixture; Mg^{2+} ions (6 mM), indicator dye.

25× BioMaster *RT-LAMP*-mix contains *RNAscribe RT* revertase and LF *Bst* DNA-polymerase in the optimal ratio for both reactions.

RNAscribe RT – genetically modified reverse transcriptase (revertase) of murine leukemia virus (*M-MuLV*). The enzyme has RNA- and DNA-dependent polymerase activity and exhibits the optimal activity at 55 °C (still active at 65 °C).

LF Bst DNA-polymerase is a large fragment of Bst (*Bacillus stearothermophilus*) polymerase (polypeptide 67 kDa), extracted from the *E.coli* strain, carrying the modified cloned gene. Enzyme has 5′-> 3′ -polymerase activity but no 5′-> 3′ nor 3′-> 5′-exonuclease activity, allowing to apply it for the colorimetric loop-mediated isothermal amplification (LAMP) performance. LF Bst DNA-polymerase exhibit high DNA-chain displacement activity and can be applied for the isothermal DNA amplification. The enzyme shows highest activity at 60-65° C temperature range.

2× RT-LAMP-Color buffer optimized for effective performance of both RT and LAMP. Additives and enhancers in its contains allow to perform effective RT-LAMP with complex and GC-rich matrixes.

The main advantage of the product consists of the easy visual detection of the reaction results. During the amplification, the reaction mixture, where the product is accumulated, change its color from red to yellow in 15-60 min, depending on the matrix concentration.

Kit contains

Catalogue number	2× RT-LAMP-Color buffer	25× BioMaster RT-LAMP-mix	DEPC treated water	Amount of 25 µl reactions
RM09-80	2 × 0.5 ml	1 × 80 µl	2 × 0.5 ml	80
RM09-400	4 × 1.25 ml	1 × 400 µl	3 × 1.8 ml	400

BioMaster RT-LAMP-Color (2×) contains:
Low-capacity buffer, 20 mM KCl, 2 mM of each nucleosidetriphosphate, 12 mM $MgCl_2$, 0.5% Tween 20, Bst LF DNA-polymerase stabilizers, indicator dye.

BioMaster RT-LAMP-mix (25×) contains:
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

- Colorimetric one-step reverse transcription (RT) и loop-mediated isothermal amplification (LAMP);
- colorimetric loop-mediated isothermal amplification with end-point detection

Application advantages

- High sensitivity (100 pg – 1 µg RNA);
- The mixture does not require additional manipulations or complex devices to visualize the reaction.

Протокол

1. De-thaw the reaction mixture and mix thoroughly. It is recommended to use ice or the precooled thermorack for the reaction preparation.
2. In PCR thin-wall tubes add the next components based on one reaction volume of 25 µl:

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

* - the mixture was designed with the low-capacity buffer, to avoid the obtainment of the false-positive results follow the next rules: sample in 1× TE-buffer can be applied in the volume no more than 1 µl, in 0,1× TE-buffer – no more than 5 µl, for the express-analysis the «HFast Lysis Buffer for DNA express-extraction» kit is recommended (FL-bio-100, FL-bio-200) up to 5 µl per reaction!

1. Mix carefully and discard the droplets, using microcentrifuge.
2. Perform the reaction at 65 °C. The reaction performance duration depends on the matrix concentration, with kit sensitivity maximum being observed at 60 min

incubation time. For the higher contrast between the negative and positive results colling the tubes for 10 to 15 min is recommended.

3. Reaction considered positive of the color of the negative control does not change (remains red), but changes in the sample (becomes yellow).

Storage conditions:

Store in place, protected from the light at -20°C – 12 month; no more than 30 freeze-thaw cycles.

Transportation conditions:

Transport in thermocontainers with cooling elements; the ambient temperature increment to the room temperature during the transportation up to 7 days is allowed.