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BioMaster RT-PCR – Standard (2×)

Cat. Number: RM02-40, RM02-200

Product description:

BioMaster RT-PCR – Standard (2×) was designed for one-tube reverse transcription and polymerase chain reaction (RT-PCR). The kit includes **2× buffer for RT-PCR-Standard** containing all the necessary components (excluding DNA template and primers); enzyme mix **BioMaster-mix** and **DEPC-treated water, DMSO** and **6×loading buffer**.

BioMaster-mix contains the optimal ratio of M-MuLV –RH to *HS-Taq* DNA polymerase for both reactions.

M-MuLV –RH is a genetically modified Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV). The enzyme exerts RNA- and DNA-dependent polymerase activity but lacks RNase H activity. M-MuLV –RH reverse transcriptase exhibits improved thermal stability and remains active up to 50°C.

HS-Taq DNA polymerase is a recombinant *Taq* DNA polymerase inactivated by specific monoclonal antibodies. The enzyme is inactive at temperatures up to 70 °C, it is activated at the first PCR cycle during a short 5-min incubation at 95 °C. Recombinant *HS-Taq* DNA polymerase catalyzes 5'→3' synthesis of DNA and possesses 5'→3' exonuclease activity of the native *Taq* DNA polymerase from *Thermus aquaticus*. Recombinant *HS-Taq* DNA polymerase is ideal for conventional PCR of the templates of up to 5 kbp.

The buffer is optimized for both efficient RT and PCR.

Product composition:

Cat. #	2× buffer for RT-PCR-Standard	BioMaster-mix	DMSO	DEPC-treated water	6×loading buffer
RM02-40	2 × 0.5 ml	1 × 80 µl	1 × 0.1 ml	2 × 0.5 ml	1 × 0.1 ml
RM02-200	4 × 1.25 ml	1 × 400 µl	1×02ml	3 × 1.8 ml	1 × 0.5 ml

2× buffer for RT-PCR-Standard contains:

100 mM Tris-HCl (pH 8.3 at 25 °C), 150 mM KCl, 0.6 mM each deoxynucleoside triphosphate, 6 mM $MgCl_2$, 8 mM DTT, enzyme stabilizers and enhancers.

BioMaster-mix contains:

50 mM Tris-HCl (pH 8.0 at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 50 % (v/v) glycerol and 0.1 % (v/v) NP-40, M-MuLV –RH reverse transcriptase and *HS-Taq* DNA polymerase.

Applications:

- Gene expression analysis;
- One-step conventional RT-PCR.

Reaction mix features

- The reaction mix is optimized for the specific and effective performance of M-MuLV –RH reverse transcriptase and *HS-Taq* DNA polymerase;
- Allows long-term storage (storage of **BioMaster RT-PCR – Standard (2×)** at room temperature for 2 days and/or multiple thawing-freezing cycles do not affect RT-PCR efficacy);

Benefits of use

- High specificity;
- High sensitivity;
- Convenient and easy-touse;
- Low pipetting error and low risk of cross-contamination;
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components in a series of experiments);
- PCR products can be further subjected to TA cloning due to deoxyadenosine overhangs at the ends of amplified DNA fragments;
- Shorter step of sample preparation for the analysis of PCR results.

Limits of use

- Not recommended for amplicons of > 5 kbp.

Protocol

Before starting to work, we recommend to get acquainted with the protocol and recommendations presented at our site: http://biolabmix.ru/catalog

1. Thaw **2× buffer for RT-PCR-Standard** and vortex thoroughly.
2. Place the thin-wall tubes in ice and add the following components considering the final volume of a reaction mixture equal to 50 µl:

Component	Volume	Final concentration
2× mix for RT-PCR-Standard	25 µl	1×
BioMaster-mix	2 µl	
Forward primer	variable	0.1 – 500 nM
Reverse primer	variable	0.1 – 500 nM
RNA template	variable	1 pg – 1 µg
Sterile water	up to 50 µl	

Note: in case of amplification of templates with complicated spatial structure, DMSO can be added in the amount of 1 to 5% of the final volume of the reaction solution. Change in Tm of the primers should be taken into account when selecting the amplification program.

Note: the volume of **BioMaster-mix** can be varied in the range of 1 to 3 µl per 50 µl reaction depending on the gene copy number and complexity.

3. Carefully vortex and discard the droplets by centrifugation.

Note: in case of using the thermal cycler without a heating lid, add a drop of mineral oil (25-35 µl) to each tube.

4. Perform PCR using recommended conditions:

Step	Temperature, °C	Incubation time	Number of cycles
Reverse transcription	45	30 min	1
Preliminary denaturation	95	5 min	1
Denaturation	95	5 – 15 sec	25 – 45
Annealing	50 – 68 (Tm-5)	5 – 20 sec	
Elongation	72	0.5-1 min/kbp	
Final elongation	72	5 – 15 min	1

Tm: template-primer duplex melting temperature, it depends on the primer structure. The following formula can be used for Tm estimation:
Tm (°C) = 2 x (A+T) + 4 x (G+C).

5. After conducting PCR, analyze amplification products by gel electrophoresis. No loading buffer is required.

Note: we recommend using 1xTAE buffer with ethidium bromide for separation of amplification products by gel electrophoresis.

Note: mobility of dyes in 0.5 – 1.5% agarose gel:

xylene cyanol	bromphenol blue	Orange G	tartrazine
10000 – 4000 bp	500-400 bp	<100 bp	<20 bp

Optimization of reaction conditions

1. The reaction volume can be varied in the range of 10 to 50 µl with proportional change in the amount of all components.
2. When using a template containing GC-rich regions and regions with complicated spatial structure, the temperature can be increased to 50 °C, and/or reagents facilitating melting of the secondary structure of the nucleic acids (e.g. DMSO) can be added.

Storage conditions: in a place protected from light at +4 ° C – 1 month; at -20 ° C - 1 year; not more than 30 thawing-freezing cycles.

Transportation Transport in thermocontainers with cooling elements; the ambient temperature increment to the room temperature during the transportation up to 10 days is allowed.