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BioMaster RT-PCR – Premium (2×)

Cat. Number: RM05-40, RM05-200

Product description:
BioMaster OT-PCR-Premium (2×) is designed for reverse transcription and polymerase chain reaction (RT-PCR) from long (up to 7 kb) and complex matrixes by one-step assay. The kit contains **2× RT-PCR Premium buffer containing** all necessary components (except enzymes, RNA matrix and primers); **BioMaster Premium mix enzyme mix, DEPC-treated Water, DMSO**, and **application buffer (6×)**.

The **BioMaster Premium Mix** contains M-MuLV -RH, *HS-Taq* DNA polymerase and *Pfu* DNA polymerase in the optimal ratio for both reactions to proceed.

M-MuLV -RH is a genetically modified reverse transcriptase (revertase) of mouse leukaemia virus (M-MuLV). The enzyme exhibits RNA- and DNA-dependent polymerase activity, but lacks the activity of RNase H. M-MuLV-RH revertase has increased thermal stability and is active up to 50 °C.

High-precision PCR is performed by a combination of two highly purified enzymes: a highly processive recombinant HS-Taq DNA polymerase and Pfu DNA polymerase with proofreading activity. The polymerase mixture is inactive at room temperature. Heating of the reaction mixture at 92-93 °C for 5 min is required to activate the enzymes.

The combination of polymerases allowed to increase the accuracy and reliability of amplification several times compared to Taq DNA polymerase. The combined use of two enzymes makes it possible to generate PCR products up to 7 bp. The products obtained with **BioMaster OT-PCR-Premium (2×)** predominantly contain 3'-dA ends, which can be used for cloning.

The **2× buffer for RT-PCR-Premium** is optimized for both efficient RT and PCR.

Product composition:

Cat. #	2× buffer for RT-PCR-Premium	BioMaster-Premium-mix	DMSO	DEPC-treated water	6×loading buffer
RM05-40	2 × 0.5 ml	1 × 80 µl	1 × 0,1 ml	2 × 0.5 ml	1 × 0,5 мл
RM05-200	4 × 1.25 ml	1 × 0.4 ml	1 × 0,2 ml	3 × 1.8 ml	1 × 1 мл

2× buffer for RT-PCR-Premium 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-Premium-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;
- Products synthesis for cloning.

Reaction mix features

- The reaction mix is optimized for the specific and effective performance of M-MuLV –RH reverse transcriptase and *mixture of polymerases*;
- Allows long-term storage (storage of **BioMaster RT-PCR – Premium (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
- Easy and convenient in use
- 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.

Limits of use

- Not recommended for primers with incomplete complementarity.

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-Premium** 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-Premium Color	25 µl	1×
BioMaster-Premium-mix	2 µl	
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
RNA template	variable	1 pg – 1 µg
Sterile water (optional)	up to 50 µl	

Note: in case of amplification of templates with complicated spatial structure, DMSO (optional) 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-Premium-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 remove 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	92-93	5 min	1
Denaturation	93	5 – 15 sec	25-50
Annealing	50 – 68 (Tm-5)	10 – 20 sec	
Elongation	68	0.5 min/kbp	
Final elongation	68	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; no 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 7 days is allowed.