Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

BioMaster RT-qPCR SYBR Blue (2×)

Cat. Number: RM04-80, RM04-400

Product description:

BioMaster RT-qPCR SYBR Blue (2×) reagent kit includes **2× buffer for RT-qPCR with SYBR** containing all the necessary components (except for RNA template and primers) including SYBR Green I intercalating dye, **BioMaster-mix** enzyme mix and **DEPC-treated water**. The kit is designed for one-step reverse transcription and real-time polymerase chain reaction (RT-qPCR).

BioMaster-mix contains the optimal ratio of M-MuLV –RH and *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 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*. The recombinant *HS-Taq* DNA polymerase is ideal for standard PCR from matrix up to 5 kbp.

2× buffer for RT-qPCR with SYBR is optimized for both efficient RT and real-time PCR. It contains additives and enhancers that enable efficient RT-qPCR of complicated and GC-rich templates. **2× buffer for RT-qPCR with SYBR** dyes blue the reaction solution which allows control when using multi-well plates.

Product composition:

Cat. #	2× buffer for RT-qPCR with SYBR	BioMaster-mix	DMSO	DEPC-treated water	Number of reactions (25 µl each)
RM04-80	2 × 0.5 ml	1 × 80 µl	0,1 ml	2 × 0.5 ml	80
RM04-400	4 × 1.25 ml	2 × 200 µl	0,2 ml	3 × 1.8 ml	400

2× buffer for RT-qPCR with SYBR 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, fluorescent dye SYBR Green I and inert dye.

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, *mixture of polymerases* and inhibitor of RNases.

Applications:
- Gene expression analysis;
- One-step real-time RT-qPCR.

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;
- The mix contains substances that allow long-term storage (storage of **BioMaster RT-qPCR SYBR Blue (2×)** at room temperature for 2 days and/or multiple thawing-freezing cycles do not affect RT-qPCR efficacy);
- The mix contains fluorescent dye SYBR Green I, which allows real-time monitoring of PCR.

SYBR Green I
SYBR Green I is a fluorescent intercalating dye for quantitative and qualitative detection of PCR products during real-time PCR. SYBR Green I provides easy and economical way for detection and quantitative assessment of PCR products during real-time PCR without a need of using specific fluorescent probes. During amplification, SYBR Green I dye penetrates into the minor groove of DNA products and emits stronger fluorescent signal than the unbound dye. Absorption and emission maxima of SYBR Green I are 494 nm and 521 nm, respectively, which enables its usage with any real-time PCR platform existing to date.

Inert dye
The inert dye included in **2× buffer for RT-qPCR with SYBR** does not reduce PCR efficiency; it facilitates monitoring of multi-well plate pipetting. Absorption maximum of the blue dye is 615 nm.

Benefits of use
- The mix is colored for easy pipetting
- High specificity
- High sensitivity (1pg – 1 µg of RNA)
- Contains an inhibitor of RNases
- 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)

Limits of use
- The presence of the fluorescent dye renders usage of fluorescent probes.

Protocol

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

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

Component	Volume	Final concentration
2× mix for RT-qPCR with SYBR	12,5 µl	1×
BioMaster-mix	1 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 25 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 0.5 to 2 µl per 25 µl reaction depending on the gene copy number and complexity.

3. Carefully vortex and remove droplets by centrifugation
4. Perform PCR using conditions presented below:

Step	Temperature, °C	Incubation time	Number of cycles
Reverse transcription	45	10-30 min	1
Preliminary denaturation	95	5 min	1
Denaturation	95	10-30 sec	25-50
Annealing	50–68 (Tm-5)	10-30 sec	
Elongation	68	0,5-1 min/kbp	
Melting curve	65–95		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. Real-time monitoring of PCR can be conducted at 72 °C in case of the absence of non-specific products (primer dimers). If non-specific products are generated with Tm1 lower than Tm2 of the target product, then reaction monitoring is performed at the temperature between Tm1 and Tm2.

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 - 3 weeks; at -20 ° C – 1 year; no more than 50 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.