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BioMaster RT-qPCR- Extreme (2×)

Cat. Number: RM01-80, RM01-400

Product description:
This **BioMaster RT-qPCR- Extreme (2×)** includes **2× RT-qPCR-Extreme buffer**, containing all necessary components (except for RNA matrix and primers); **BioMaster Mix Extreme** and **DEPC-treated water**. The kit is designed for reverse transcription and real-time polymerase chain reaction (real-time RT-PCR) with fluorescent probes using a one-tube assay.

25× BioMaster-Extreme-mix contains the optimal ratio of RNAscribe RT reverse transcriptase to *HS-Taq* DNA polymerase for both reactions.

RNAscribe RT Reverse Transcriptase (RT) is a genetically modofied MMLV (Moloney Murine Leukemia Virus) based Reverse Transcriptase. This is an RNA-directed DNA polymerase that can synthesize a complementary DNA strand from ssRNA or ssDNA and is active over a broad range of reaction temperatures from 37°C-60°C. *RNAscribe RT* is a robust enzyme for RNA detection and has enhanced stability at room temperature with no activity loss for up to 1 month. This RT contains a functional RNase H domain which can increase the sensitivity of RT-qPCR (quantitative reverse transcription PCR).

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

Product composition:

Cat. #	2× buffer for RT-qPCR	25× BioMaster-Extreme-mix	DEPC-treated water	Number of reactions (25 µl each)
RM01-80	2 × 0.5 ml	1 × 80 µl	2 × 0.5 ml	80
RM01-400	4 × 1.25 ml	1 × 400 µl	3 × 1.8 ml	400

2× buffer for RT-qPCR contains:
100 mM Tris-HCl (pH 8.5 at 25 °C), 150 mM KCl, 0.8 mM each deoxynucleoside triphosphate, 8 mM $MgCl_2$, 10 mM TCEP, enzyme stabilizers and enhancers.

25× BioMaster-Extreme-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, *RNAscribe RT* reverse transcriptase, highly-processive HS-*Taq* DNA polymerase and inhibitor of RNases.

Applications:

- Gene expression analysis
- One-step conventional RT-qPCR

Reaction mix features

- The mixture is optimized for specific and efficient operation of *RNAscribe* RT revertase and *HS*-Taq DNA polymerase
- Provides long term storage (**BioMaster OT-PCR-RV-Extreme (2×)** storage for 10 days at room temperature and repeated freezing and thawing does not reduce the efficiency of RT-PCR).
- The mixture does not contain dyes, making it multipurpose.

Benefits of use

- Revertase can work at 60 °C;
- High specificity;
- High sensitivity;
- Simple and convenient application;
- Reduced pipetting error and cross-contamination possibility;
- Standardized conditions of the same-type reactions (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.

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-qPCR** and mix thoroughly.
2. Place the thin-wall tubes in ice and add the following components considering the final volume of a reaction mixture equal to 25 µl:

Component	Volume	Final concentration
2× buffer for RT-qPCR	12,5 µl	1×
25× BioMaster-Extreme-mix	1 µl	
Forward primer	variable	0.1 – 500 nM
Reverse primer	variable	0.1 – 500 nM
Probe	variable	0.1 – 300 nM
RNA template	variable	1 pg – 1 µg
Sterile water (optional)	up to 25 µl	

Note: in case of amplification of templates with complicated spatial structure, DMSO (is not included) can be added in the amount of 1 to 5% of the final volume of the reaction solution. Changes in Tm of the primers should considered when selecting the amplification program.

Note: the volume of **25× BioMaster-Extreme-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. Vortex carefully and remove droplets by centrifugation.

4 Perform PCR using the recommended conditions presented below:

Step	Temperature, °C	Incubation time	Number of cycles
Reverse transcription	50-55	10-20 min	1
Preliminary denaturation	95	5 min	1
Denaturation	95	5-10 sec	30–50
Annealing	50 – 68 (Tm-5)	10 - 20 sec	
Elongation	72	0,5-1 min/kbp	

Or:

Step	Temperature, °C	Incubation time	Number of cycles
Reverse transcription	50-55	10-20 min	1
Preliminary denaturation	95	5 min	1
Denaturation	95	5-10 sec	30–50
Annealing/elongation	50 – 68	40-60 sec	

5 PCR results are displayed as amplification curves.

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 60 °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.