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BioMaster RT-PCR – Extra (2×)

Cat. Number: RM06-40, RM06-200

Product description:

BioMaster RT-PCR-Extra (2×) is designed for reverse transcription and polymerase chain reaction (RT-PCR) from long (up to 9 kb) and complex matrices using a one-step method. The kit contains **2× RT-PCR Extra buffer** containing all necessary components (except enzymes, RNA matrix and primers); **BioMaster Extra-mix enzyme mix**, **DEPC-treated Water**, **DMSO** and **application buffer (6×)**.

BioMaster Extra-mix contains *RNAscribe RT* revertase, *HS-Taq* DNA polymerase and *Pfu* DNA polymerase in optimal ratios for both reactions to proceed.

RNAscribe RT is a genetically modified reverse transcriptase (revertase) of mouse leukaemia virus (M-MuLV). The enzyme exhibits RNA- and DNA-dependent polymerase activity and shows optimal activity at 55 °C (active up to 65 °C). The enzyme is capable of first strand of cDNA synthesis up to 9 bp in length and incorporating modified bases. Its fast reaction rate allows the synthesis to be performed in just 15 minutes, and the enzyme's high operating temperature (up to 65 °C) ensures high yield and specificity of the reaction.

High-precision PCR is performed by a combination of two highly purified enzymes: high-processive recombinant HS-Taq DNA polymerase and Pfu DNA polymerase with proofreading activity. The polymerase mixture is inactive at room temperature. Heating 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 in several times in comparison with Taq DNA polymerase. The combined use of the two enzymes makes it possible to generate PCR products up to 9 tbp. The products obtained with **BioMaster RT-PCR-Extra (2×)** predominantly contain 3'-dA ends, which can be used for cloning.

The buffer is optimised for efficient flow of both OT and PCR.

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

Product composition:

Cat. #	2× buffer for RT-PCR-Extra	BioMaster-Extra-mix	DMSO	DEPC-treated water	6×loading buffer
RM06-40	2 × 0.5 ml	1 × 80 µl	1 × 0.1 ml	2 × 0.5 ml	0.1 ml
RM06-200	4 × 1.25 ml	1 × 0.4 ml	1×0.2 ml	3 × 1.8 ml	0.5 ml

Product composition

2× buffer for RT-PCR-Extra contains:
100 mM Tris-HCl (pH 8.3 at 25 °C), 150 mM KCl, 0.8 mM each deoxynucleoside triphosphate, 6 mM $MgCl_2$, 1 mM TCEP, enzyme stabilizers and *mixture of polymerases*.

BioMaster-Extra-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, RNAse inhibitor, *RNAscribe RT* reverse transcriptase, *Pfu* and *HS-Taq* DNA polymerases.

Applications:
- Gene expression analysis
- One-step high-precision RT-PCR
- Product generation for cloning

Reaction mixture characteristics
- The mixture is optimised for specific and efficient operation of *RNAscribe RT* revertase, *HS-Taq* and *Pfu* DNA polymerases;
- Provides long-term storage (BioMaster RT-PCR-Extra (2×) storage for 5 days at room temperature and repeated freeze-thawing does not reduce RT-PCR efficiency);

Benefits of use
- cDNA synthesys at high temperatures (up to 65 °C);
- High specificity;
- High fidelity PCR (higher, than *Taq* DNA polymerase);
- 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-Extra** 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-Extra	25 µl	1×
BioMaster-Extra-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: the volume of **BioMaster-Extra-mix** can be varied in the range of 1 to 4 µl per 50 µl reaction depending on the gene copy number and complexity.

3. Carefully vortex and discard 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	55*	30-50 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

* - revertase works without significant loss of activity up to 65° C, it may be necessary to lower the temperature of the RT step to obtain long fragments.

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.

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 65 °C, and/or reagents

facilitating melting of the secondary structure of the nucleic acids (e.g. DMSO) can be added.

Storage conditions: 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.

3.