

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 HS-*Taq* PCR (2×)

Cat. number MH010-200, MH010-1020

Product description:

BioMaster HS-Taq PCR (2*) includes 2× **BioMaster HS-Taq PCR (2*)** reaction mix, 50 mM MgCl₂, and 6× loading buffer. The 2× **BioMaster HS-Taq PCR (2*)** reaction mix has been developed for a PCR analysis of a wide range samples. **BioMaster HS-Taq PCR (2*)** contains all of the necessary components (except for DNA template and primers) for a PCR reaction:

- highly processive recombinant HS-Taq DNA polymerase;
- · deoxynucleoside triphosphate mix;
- PCR buffer;
- Mg²⁺.

The mix is optimized for the efficient performance and reproducible hot-start PCR. **BioMaster HS-Taq PCR (2x)** contains additional components that increase the half-life and processivity of HS-Taq DNA polymerase enhancing its stability during PCR. **BioMaster HS-Taq PCR (2x)** is chemically stable, inert and does not interfere with optimal annealing temperature or the parameters of template melting.

DNA polymerase included in the mix is inactive at room temperature and requires preheating at 95 $^{\circ}$ C for 5 min. Additional MgCl₂ solution allows easy optimization of the reaction mix for each individual primer/template system. Use of the mix helps saving experimental time and minimizes contamination risk due to reduced number of pipetting steps.

Product composition

Cat.#	BioMaster HS- Taq PCR (2×)	50 mM MgCl ₂	Water	6× loading buffer	Number of reactions (50 µl)
MH010-200	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	1 × 1 ml	200
MH010-1020	17 × 1.5 ml	1 × 1.8 ml		2 × 1.8 ml	1020

BioMaster HS-Taq PCR (2*) contains:

100 mM Tris-HCl (pH 8.5 at 25 °C) 100 mM KCl, 0.4 mM of each deoxynucleoside triphosphate, 4 mM MgCl₂, 0.06 U/ μ l Taq DNA polymerase, 0.2% Tween 20, stabilizers of HS-Taq DNA polymerase.

Applications:

- Hot-start PCR;
- High-throughput PCR;
- · Conventional PCR with high reproducibility;
- Generation of PCR products for TA cloning;
- RT-PCR.

Taq DNA Polymerase features

Recombinant HS-Taq DNA polymerase possesses 5' \rightarrow 3' DNA-dependent polymerase activity and 5' \rightarrow 3' exonuclease activity of native Taq DNA Polymerase from *Thermus aquaticus*. The rate of DNA synthesis by Taq polymerase depends on the complexity of DNA template and is approximately 1 kbp/min. Recombinant HS-Taq DNA Polymerase is ideal for conventional PCR of templates up to 5 kbp.

Reaction mix features

- The reaction mix is optimized for specific performance of HS-Taq DNA polymerase, long-term storage (the storage of BioMaster HS-Taq PCR (2x) at room temperature for 30 days does not affect PCR efficiency), multiple thawing-freezing cycles;
- The mix contains components increasing density of sample solution for easy gel loading;
- The mix does not contain substances, interfering with reaction course visual monitoring and changes in sample fluorescence.

Benefits of use:

- Hot-start enzyme increases specificity, sensitivity and reaction yield.
- HS-*Taq* DNA polymerase activation requires no more than 5 min of heating.
- Reducuction of reaction preparation time.
- Low chance of contamination during preparation of PCR solution.
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components from experiment to experiment).
- Can be applied for a wide range of PCR types.
- PCR products can be further subjected to TA cloning due to the presence of deoxyadenosine overhangs in amplified DNA.

Limits of use

• Not recommended to use for amplicons >5 kbp

Amplification protocol

- 1. Thaw the reaction mixture and mix thoroughly.
- 2. Add the following components into the thin-wall PCR tubes considering the final volume of a reaction mixture being 50 μ l:

Component	Volume	Final concentration
BioMaster HS-Taq PCR (2×)	25	1×
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
DNA template	variable	1 pg - 1 μg
Sterile water	up to 50 μl	

3. Carefully vortex and remove droplets by centrifugation.

Note: in case if a thermal cycler is not equipped with a heated lid, add a droplet (25–35 μ l) of mineral oil in each tube.

4. Perform PCR using the assay recommended below:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	3 - 5 min	1
Denaturation	95	10 – 20 sec	
Annealing	50 – 68 (Tm-5)	10 - 20 sec	25-50
Elongation	72	0,5 min/kbp	
Final elongation	72	5 – 15 min	1

Tm – template/primer melting temperature, depends on primer structure. The following formula can be used for approximate estimation of Tm: Tm ($^{\circ}$ C) = 2 x (A+T) + 4 x (G+C).

5. After performing PCR, analyze amplification products by gel electrophoresis. Samples are loaded on gel without additional loading buffer.

Note: we recommend using 1x TAE buffer with ethidium bromide for visualizing PCR 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

Storage conditions: in a place protected from light at +25 ° C - 7 days; at +4 ° C - 4 months; at -20 ° C - 18 months; not 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 10 days is allowed.