Product information

BioMaster HS-*Taq* PCR Master Mix (2×)

Product description

BioMaster HS-*Taq* PCR Master Mix (2×) includes 2× ***BioMaster HS-Taq PCR Master Mix*** reaction mix, 50 mM $MgCl_2$ and sterile water. The mix has been developed for PCR analysis of a large number of samples. ***BioMaster HS-Taq PCR Master Mix (2×)*** contains all components (except for DNA template and primers) needed for a PCR reaction:

- highly processive recombinant HS-*Taq* DNA polymerase;
- deoxynucleoside triphosphate mix;
- 2× PCR buffer;
- Mg^{2+}.

The mix is optimized for performing efficient and reproducible hot-start PCR. ***BioMaster HS-Taq PCR Master Mix (2×)*** contains additional components increasing the half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. ***BioMaster HS-Taq PCR Master Mix (2×)*** 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 °C for 5 min. Additional $MgCl_2$ 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 Master Mix (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 Master Mix (2×) contains*:**

100 mM Tris-HCl (pH 8.5 at 25 °C) 100 mM KCl, 0.4 mM of each deoxynucleoside triphosphate, 4 mM $MgCl_2$, 0.06 U/µl *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'→3' DNA-dependent polymerase activity and 5'→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 Master Mix (2×)*** 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 that interfere with optical monitoring of a reaction course and changes in sample fluorescence.

Benefits of use:

- Hot-start enzyme increases specificity, sensitivity and reaction yield
- HS-*Taq* DNA polymerase activation requires 5 min heating
- Reduced 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 various range of PCR applications
- 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
- Not recommended to use with fluorescently labeled probes

Amplification protocol

1. Defrost the reaction mixture and stir thoroughly.
2. Add the following components into the thin-wall PCR tubes considering the final volume of a reaction mixture equal to 50 µl:

Component	Volume	Final concentration
BioMaster HS-Taq PCR Master Mix (2×)	25	1×
Forward primer	variable	0.1 – 300 nM
Reverse primer	variable	0.1 – 300 nM
DNA template	variable	10 pg – 1 µg
Sterile water	up to 50 µl	

3. Gently 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 µl) of mineral oil in each tube.

4. Perform PCR using temperature conditions recommended below:

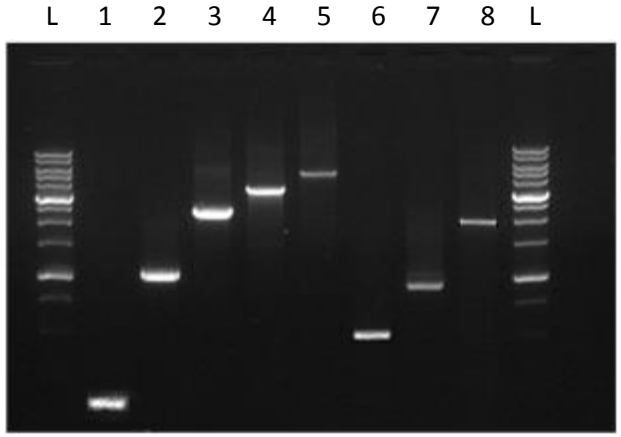
Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5-7 min	1
Denaturation	95	15 – 30 sec	25 - 40
Annealing	50 – 68 (Tm-5)	15 - 30 sec	
Elongation	72	1 min/kbp	
Final elongation	72	5 – 15 min	1

Tm – template/primer melting temperature, depends on primer structure. The following formula can be used for approximate estimation of Tm: Tm (°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

DNA amplification using *BioMaster HS-Taq PCR Master Mix (2×)*



Lane L – molecular DNA marker of 250 to 10000 bp. Lanes 1-5 – amplification of phage λ DNA fragments of 175, 1000, 2000, 3500 and 5000 bp, respectively. Lanes 6-8 – amplification of human genomic DNA fragments of 500, 900 and 2000 bp, respectively.

Storage and transportation: at -20 °C; not more than 50 thawing-freezing cycles.

Storage terms: 1 year (under proper storage and transportation conditions).

Recommendations for avoiding contamination during PCR
Over 10 million copies of DNA template are processed during PCR. Therefore, it is important to prevent the possibility of contamination with other templates and amplicons that are present in laboratory. Here are general recommendations for reducing the risk of contamination:

- Preparation of DNA samples, preparation of reaction solutions, amplification and analysis of PCR products should be carried out in different territorial areas.
- Prepare reaction solutions in PCR laminar flow cabinet equipped with UV lamp.
- Use new pair of gloves when purifying DNA and preparing mixtures and solutions.
- Use reagents designed specifically for PCR. Use pipette tips with integrated aerosol filter when preparing DNA samples and reaction solutions.
- For verification of the absence of contamination, prepare a mixture sample without DNA template (negative control).

Recommendations for primer selection
For primer design, use well-established programs like Primer3
http://biotools.umassmed.edu/bioapps/primer3_www.cgi and follow the basic principles:

- Primer length usually falls in the range of 18 – 22 nucleotides.
- Difference in melting temperatures (Tm) of the two primers shouldn't exceed 3 °C.
- Optimal GC composition of the primers is 40 – 60%. Theoretically, G and C nucleotides should be evenly distributed over the whole length or primer.
- Avoid the presence of ≥3 G or C nucleotides at the 3' terminus of primer in order to prevent risk of nonspecific annealing.
- If possible, primer should end with G or C nucleotide at 3' end.
- Avoid using primers with self-complementary regions, primers complementary to each other and primer repeats for preventing formation of hairpin structures and primer-dimers.
- Make sure that there are no unwanted complementary regions between primers and DNA template.
- When selecting degenerated primers, they should contain at least three conserved nucleotides at the 3' end.

Components of reaction solution
DNA template
Optimal amount of DNA template per 50 µl reaction solution is 0.01 – 1 ng in case of using plasmid or phage DNA and 0.1 – 1 µg in case of genomic DNA. Higher amounts of template increase the risk of formation of non-specific amplification products, low amounts of template reduce accuracy of amplification. All conventional techniques of DNA purification can be applied for the preparation of a studied sample. It should be mentioned that trace amounts of certain agents used for the isolation and purification of DNA, such as phenol, EDTA and proteinase K, can inhibit DNA polymerase. Precipitation and repeated washing with 70% ethanol usually removes trace contaminants from DNA sample.

Primers
Recommended concentrations of PCR primers are in the range of 0.1 – 0.6 μM. Excessive concentration of primers increases the chance of non-specific binding to the template and formation of alternative PCR products.
For degenerated primers and primers utilized for PCR of long fragments, we recommend using higher concentrations in the range of 0.3 – 1 μM.

Characteristics of amplification steps
Initial DNA denaturation and enzyme activation
It is very important to achieve complete denaturation of DNA template at the beginning of PCR which provides its efficient use in the first amplification cycle. If GC composition of the template is 50% or less, initial denaturation at 95 °C for 1-3 min will be enough.

Denaturation
Standard time of denaturation per cycle is considered to be 30 sec at 95 °C. For GC-rich DNA templates this step can be extended to 3-4 minutes.

Primer annealing
Annealing temperature for primers should be 5 °C lower than their melting temperature (Tm). Conventional annealing time is 30 sec. In case if accumulation of non-specific PCR products takes place, the annealing temperature should be optimized by stepwise 1-2 °C increase.

Elongation
Optimal efficiency of *Taq* DNA polymerase is observed in the temperature range of 70 – 75 ° C. The rate of synthesis by *Taq* DNA polymerase ranges from 30 to 60 bp per second depending on template complexity. In the case of using long templates (>2 kbp), it is recommended to estimate elongation time based on the ratio 1 min/kb.

Number of cycles
If there is less than 10 copies of DNA template available per reaction, then efficient amplification requires not less than 40 cycles. A total of 25 – 35 cycles is enough for higher amount of template.

Final elongation
When the last cycle is finished, it is recommended to incubate PCR solution for further 5 – 15 min at 72 °C for complete synthesis of the products. If PCR product is to be further cloned into TA vector, final elongation should be extended to 30 min in order to achieve maximal efficiency of formation of 3'-dA ends of PCR products.

Exodeoxyribonuclease activity
DNA was found stable after incubation of 1 μg fragment of phage lambda DNA in the presence of 25 μl of ***BioMaster HS-Taq PCR Master Mix (2×)*** in 50 μl reaction solution at 37 °C and 70 °C for 4 h.

Ribonuclease activity
Absence of ribonuclease activity was confirmed after incubation of 1 μg of 5'-[P^{32}]-labeled RNA fragment in the presence of 25 μl of ***BioMaster HS-Taq PCR Master Mix (2×)*** in 50 μl reaction solution at 37 °C for 4 h.

Functional analysis
BioMaster HS-Taq PCR Master Mix (2×) was tested during amplification of fragments of phage lambda DNA of various length and human genomic DNA.

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