Product information

BioMaster Extended HS-*Taq* PCR Kit

Product description

BioMaster Extended HS-*Taq* PCR Kit contains recombinant HS-*Taq* DNA polymerase, three reaction buffers and other components necessary for PCR, except for DNA template and primers. The kit includes:

- HS-*Taq* DNA polymerase (5 U/µl);
- 10× PCR buffer;
- 5× Green buffer;
- 5× GC buffer;
- 50 mM $MgCl_2$ (separate tube);
- 50× dNTP mix;
- 6× loading buffer.

HS-*Taq* DNA polymerase is a recombinant *Taq* DNA polymerase inactivated by specific monoclonal antibodies. HS-*Taq* DNA polymerase is inactive at temperatures up to 70 °C, which allows to avoid the formation of non-specific products and primer-dimers at low temperatures during preparation of PCR solution. The activation is achieved during 5-minute incubation at the first cycle of amplification at 95 °C. Recombinant *Taq* DNA polymerase has 5´-3´ DNA-dependent polymerase activity and 5´-3´ exonuclease activity of native *Taq* DNA polymerase form *Thermus aquaticus*. The rate of DNA synthesis by *Taq DNA* polymerase depends on the complexity of DNA template and is approximately 1 kbp/min. Recombinant form of the enzyme is ideal for conventional PCR of templates up to 5 kbp.

10× PCR buffer is optimized for most types of PCR including real-time PCR with intercalating dyes or fluorescent probes. The buffer is chemically stable, inert and does not affect the optimal temperature of primer annealing or characteristics of template melting.

5× Green buffer contains marker dyes (blue-green and yellow) and additives that increase solution density for easy gel loading. The buffer is developed for conventional PCR with further product yield assessment by gel electrophoresis. The buffer is chemically stable, inert and does not affect the optimal temperature of primer annealing or characteristics of template melting.

5× GC buffer is developed for amplification of GC-rich DNA regions and/or templates with complicated spatial structure. The buffer is chemically stable and contains substances that change primer annealing temperature and characteristics of template melting.

The buffers contain additives increasing half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. Each buffer contains 1.5 mM $MgCl_2$ (per 1× buffer solution).

Additional tubes of 50 mM $MgCl_2$ and 50× dNTP mix included in the kit allow easy optimization of PCR conditions for individual primer/template system.

Product composition

Component	Cat. # (activity units)	
	KH018-500 (500 U)	K0H18-2500 (2500 U)
HS-Taq* DNA polymerase, 5 U/µl	1 × 100 µl	2 × 250 µl
10× PCR buffer	1 × 1.5 ml	3 × 1.8 ml
5× Green buffer	2 × 1.5 ml	5 × 1.8 ml
5× GC buffer	2 × 1.5 ml	5 × 1.8 ml
50 mM $MgCl_2$	1 × 1 ml	2 × 1 ml
50× dNTP mix (10 mM each)	2 × 200 µl	2 × 800 µl
6× loading buffer	1 × 1 ml	1 × 1.8 ml

* One activity unit is the amount of the enzyme required to catalyze incorporation of 10 nmol of dNTP into an acid-insoluble product in 30 min at 74 °C. Reaction conditions: 50 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM NaCl, 10 mM $MgCl_2$, 200 mM dATP, 200 mM dCTP, 200 mM dGTP, 50 mM [^{3}H] dTTP, 0.25 mg/ml of activated calf thymus DNA.

***Storage buffer*:**
50 mM Tris-HCl (pH 8.0 at 25 °C), 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% (v/v) glycerin, and 1% (v/v) Triton X-100.

***10× PCR buffer*:**
100 mM Tris-HCl (pH 8.5 at 25 °C), 500 mM KCl, 15 mM $MgCl_2$, 0.5% (v/v) Tween 20, stabilizers of *Taq* DNA polymerase.

***5× Green buffer*:**
50 mM Tris-HCl (pH 8.5 at 25 °C), 250 mM KCl, 7.5 mM $MgCl_2$, 0.5% (v/v) Tween 20, stabilizers of *Taq* DNA polymerase, marker dyes.

***5× GC buffer*:**
100 mM Tris-HCl (pH 8.5 at 25 °C), 250 mM KCl, 7.5 mM $MgCl_2$, 0.5% (v/v) Tween 20, DMSO, stabilizers of *Taq* DNA polymerase.

***Applications*:**

- Hot-start PCR;
- High-throughput PCR;
- Conventional PCR with high reproducibility;
- Generation of PCR products for TA cloning;
- Second step of RT-PCR;
- PCR of GC-rich and DNA regions and regions with complicated structure.

Limits of use

- Not recommended to use for amplicons of >5 kbp.

Inhibition and inactivation
Inhibitors: ionic detergents (sodium deoxycholate, sarkosyl and sodium dodecyl sulfate (SDS) at concentrations above 0.06, 0.02 and 0.01%, respectively).
Inactivated by phenol/chloroform extraction.

Протокол выполнения амплификации
Приготовьте несколько параллельных реакций и минимизируйте возможную ошибку
Примечание: для разделения продуктов реакции электрофорезом мы рекомендуем использовать 1xTAE буфер с бромистым этидием.

Amplification protocol

Prepare several parallel reactions and minimize possible pipetting error: mix water, buffer, dNTP mix, primers and HS-*Taq* DNA polymerase. Prepare reaction solution by multiplying the total volume by the number of reactions required plus one additional reaction to cover pipetting losses. Aliquot PCR solution to individual PRC tubes, then add DNA template.

1. Defrost the reaction mix and stir gently.
Note: in case of precipitate formation, heat the tube at 50 °C and stir till complete dissolution.

2. Add the following components into thin-wall PCR tubes considering that the final volume of a reaction mixture is 50 µl:

Component	Volume	Final concentration
10 (5)× PCR buffer	5 (10) µl	1×
50× dNTP mix	1 µl	0.2 mM each
Forward primer	variable	0.1 – 300 nM
Reverse primer	variable	0.1 – 300 nM
DNA template	variable	1 pg – 1 µg
***HS-Taq* DNA polymerase, 5 U/µl**	variable	1-5 U
Sterile water	up to 50 µl	

3. Gently vortex and collect all droplets from the tube walls by brief centrifuging.
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 conditions recommended below:

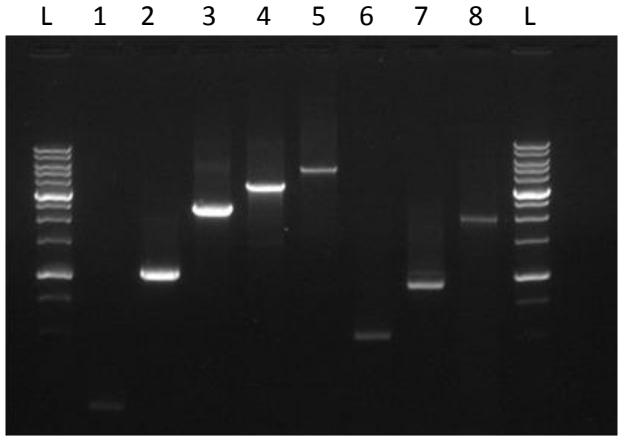
Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	15 – 30 sec	25 - 40
Annealing	50 – 68 (Tm-5)	15 - 30 sec	
Elongation	72	1 min/kbp	
Final elongation	72	5 – 15 min	1

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).
Tm (°C) = 2 x (A+T) + 4 x (G+C).

Note: in case of using 5× GC buffer, it is possible, that Tm should be 2-5 °C lower due to the presence of substances affecting primer/матрица.

5. Method of analysis of PCR results depends on the type of reaction (conventional or real-time PCR). When uisng 5× Green buffer, analysis is conducted by electrophoresis without additional preparation.

DNA amplification using 5× Green buffer of BioMaster Extended HS-*Taq* PCR Kit



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.

Mg^{2+} concentration

Change in concentration of Mg^{2+} ions can have a significant impact on PCR efficiency and specificity. These ions are necessary for the performance of *Taq* DNA polymerase. They also bind with deoxyribonucleotides in the ratio 2:1. Therefore, additional optimization of Mg^{2+} concentration may be necessary if dNTP concentration is altered in the reaction solution. The recommended concentration for Mg^{2+} is 1-5 mM. In case if Mg^{2+} concentration is too small, the yield of PCR product will be reduced. On the other hand, the formation of non-specific products and decreased PCR specificity can take place at high concentration of Mg^{2+} is observed the appearance of nonspecific PCR products and decrease precision. If DNA sample DNA contains EDTA or other substances, chelating metals, concentration of Mg^{2+} should be increased proportionally in PCR mixture (binding to EDTA occurs at the ratio 1:1).

dNTP

Recommended final concentration of each dNTP is 0.2 mM. Several PCR techniques require using higher dNTP concentrations. In such cases, it should be taken into account that dNTP bind Mg^{2+} and its concentration should be corrected. It is important that all of the four nucleotides (dATP, dCTP, dGTP and dTTP) should present in the reaction solution at equimolar concentrations.

Primers

Recommended concentrations of PCR primers are in the range of 0.1 – 1 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

No DNA degradation was noted after incubation of 1 µg fragment of phage lambda DNA in the presence of 5 U/µl of HS-*Taq* DNA polymerase 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 5 U/µl of HS-*Taq* DNA polymerase in 50 µl reaction solution at 37 °C for 4 h.

Functional analysis

BioMaster Extended HS-*Taq* PCR Kit was tested during amplification of fragments of phage lambda DNA of various length and human genomic DNA.

Biolabmix Ltd.
630090, Novosibirsk
Inzhenernaya str., 28
Tel. +7 (383) 363-51-91
http://www.biolabmix.ru