Product information

BioMaster HS-qPCR SYBR Blue (2×)

Product description

BioMaster HS-qPCR SYBR Blue (2×) contains 2× ***BioMaster HS-qPCR SYBR Blue (2×)*** reaction mix, 100 mM $MgCl_2$ solution and sterile water. ***BioMaster HS-qPCR SYBR Blue (2×)*** is developed for quantitative real-time PCR with fluorescent dye SYBR Green I. В состав ***BioMaster HS-qPCR SYBR Blue (2×)*** contains every component (except for DNA template and primers) necessary for PCR:

- highly-processive recombinant HS-*Taq* DNA polymerase
- deoxynucleoside triphosphate mix
- PCR buffer
- Mg^{2+} (3 mM)
- SYBR Green I
- Inert dye.

The mix is optimized for conducting efficient and reproducible hot-start real-time PCR of genomic, plasmid and viral DNA samples. The solution contains substances that increase half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. ***BioMaster HS-qPCR SYBR Blue (2×)*** includes components that influence primer annealing temperature and characteristics of template melting thus enabling to increase the specificity of PCR and use templates with complicated spatial structure. DNA polymerase included in the mix is inactive at room temperature, its activation requires preheating at 95 °C for 5 min. Blue hue of the reaction solution provided by the inert dye allows control when using multi-well plates. Use of the kit saves time and minimizes contamination risk due to reduced number of pipetting steps. Low concentration of Mg^{2+} in the reaction mix and additional solution of 50 mM $MgCl_2$ included in the kit allow optimizing PCR conditions for individual primer pairs.

Product composition

Cat. #	*BioMaster HS-qPCR SYBR Blue (2×)*	50 mM $MgCl_2$	Water	Number of reactions (50 µl)
MHC030-200	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	200
MHC030-1020	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	1020

***BioMaster HS-qPCR SYBR Blue (2×) contains*:**

100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, 0.4 mM each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 0.06 U/µl *Taq* DNA polymerase, 0.025% Tween 20, stabilizers of HS-*Taq* DNA polymerase, SYBR Green I, inert dye.

Applications:

- Real-time PCR with intercalating dye SYBR Green I;
- Conventional PCR;
- High-throughput PCR;
- Genotyping.

Taq DNA Polymerase features

Recombinant *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 and real-time PCR.

SYBR Green I

SYBR Green I is a fluorescent intercalating dye for quantitative and qualitative detection of PCR products during real-time PCR. SYBR Green I provides easy and economical way for detection and quantitative assessment of PCR products during real-time PCR without a need of using specific fluorescent probes. During amplification, SYBR Green I dye penetrates into the minor groove of DNA products and emits stronger fluorescent signal than unbound dye. Absorption and emission maxima of SYBR Green I are 494 nm and 521 nm, respectively, which enables to use it for every real-time PCR platform existing to date.

Inert dye

The inert dye included in ***BioMaster HS-qPCR SYBR Blue (2×)*** does not reduce PCR efficiency; it facilitates monitoring of multi-well plate pipetting. Absorption maximum of a blue dye is 615 nm.

Reaction mix features

- The mix is optimized for real-time PCR;
- The mix contains substances that increase storage time (storage of **BioMaster HS-qPCR SYBR Blue (2×)** for a month at room temperature does not reduce PCR efficiency) and allow multiple freezing-thawing cycles.

Benefits of use

- Enzyme with hot start capability increases reaction specificity and sensitivity
- HS-*Taq* DNA polymerase activation requires not more than 5 min heating
- High selectivity and reaction yield
- The mix is colored for easy pipetting
- Reduced preparation time
- Low contamination risk when mixing PCR components
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components in a series of experiments)

Limits of use

- Not recommended to use for real-time PCR with fluorescently labeled probes. **BioMaster HS-qPCR (2×)** should be used for such purposes.

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-qPCR SYBR Blue (2×)	25	1×
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
DNA template	variable	10 pg – 1 µg
Sterile water	up to 50 µl	

3. Gently vortex and remove droplets by centrifugation.
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 sec	25 - 40
Annealing	50 - 68	10-30 sec	
Elongation	58 - 72	30-60 sec	
Melting curve (recommended)			1

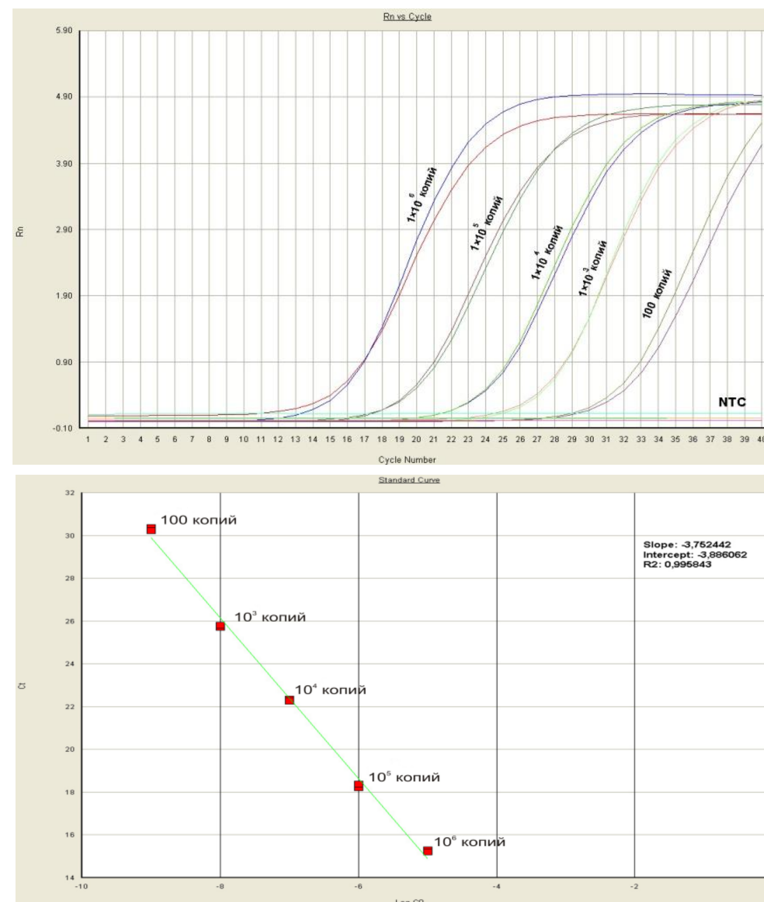
5. PCR result is displayed as amplification curves.

Note: Monitoring of real-time PCR can be conducted at 72 °C in case of the absence of non-specific products (primer-dimers). In case if non-specific products are formed with Tm_1 lower than Tm_2 of the main product, monitoring should be performed at temperatures between Tm_1 and Tm_2.

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

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

Amplification curves and standard curve obtained during real-time PCR using BioMaster HS-qPCR SYBR Blue (2×)



Amplification of MART1 gene fragment of recombinant plasmid in 10-fold serial dilutions. Amplicon length is 220 bp. Reaction was performed at ABI 7500 thermal cycler. Amplification curves and standard curve show area of system linearity. NTC – negative control.

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 – 30 nucleotides.
- Melting temperature is 58-65 °C. Difference in melting temperatures (Tm) of the two primers shouldn't exceed 3 °C.
- Recommended length of amplicon for real-time PCR is 50 – 200 bp.
- 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.
- Check your primers using BLAST.

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.

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.

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.

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).

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 seconds 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.

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 stable after incubation of 1 µg fragment of phage lambda DNA in the presence of 25 µl of ***BioMaster HS-qPCR SYBR Blue (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-qPCR SYBR Blue (2×)*** in 50 µl reaction solution at 37 °C for 4 h.

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