Product information

BioMaster UDG HS-qPCR SYBR Blue (2×)

Product description

BioMaster UDG HS-qPCR SYBR Blue (2×) includes 2× ***BioMaster UDG HS-qPCR SYBR Blue (2×)*** reaction mix, 50 mM $MgCl_2$ solution and sterile water. The mix has been developed for quantitative real-time PCR with fluorescent dye SYBR Green I. ***BioMaster UDG HS-qPCR SYBR Blue (2×)*** contains all of the components necessary for PCR (except for DNA template and primers):

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

The mix is optimized for efficient and reproducible hot-start real-time PCR of genomic, plasmid and viral DNA samples. The solution contains additives increasing the half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. ***BioMaster UDG HS-qPCR SYBR Blue (2×)*** reaction mix includes components that influence primer annealing temperature and characteristics of template melting, which enhance PCR specificity and allow usage of templates with complicated spatial structure. N-uracil-DNA glycosylase and dUTP (with the dUTP/dTTP ratio of 1:1) provide reliable protection against amplicon carryover between the reaction solutions (cross-contamination). 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 due to the presence of 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 Mg2+ concentration in the reaction mix and additional solution of 50 mM $MgCl_2$ included in the kit allow optimizing PCR conditions for individual primer-template system.

Product composition

Cat. #	*BioMaster UDG HS-qPCR SYBR Blue (2×)*	50 mM $MgCl_2$	Water	Number of reactions (50 µl)
MHC031-200	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	200
MHC031-1020	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	1020

BioMaster UDG HS-qPCR SYBR Blue (2×) includes:

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

Applications:

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

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 *Taq* DNA Polymerase is ideal for conventional 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 intercalates into the minor groove of DNA products and emits stronger fluorescent signal than the unbound dye. Absorption and emission maxima of SYBR Green I are 494 nm and 521 nm, respectively, which enables using it for every real-time PCR platform existing to date.

Inert dye

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

Reaction mix features

- The reaction mix is inactive at room temperature due to the hot-start technology and is activated by a 5-minute incubation at 95 °C;
- The presence of dUTP guarantees incorporation of uridine into each synthesized DNA strand; UDG is capable of eliminating uracil from single- and double-stranded DNA molecules;
- The mix is optimized for specific performance of HS-*Taq* DNA polymerase, long-term storage (the storage of ***BioMaster UDG HS-qPCR SYBR Blue (2×)*** at room temperature for 7 days does not affect PCR efficiency), multiple thawing-freezing cycles.

Benefits of use

- Hot-start enzyme increases reaction specificity, sensitivity and reaction yield
- Activation of HS-*Taq* DNA polymerase requires not more than 5-minute heating
- The mix is colored for easy pipetting
- Reduced preparation time
- Prevents reamplification of carryover PCR products
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components from experiment to experiment)

Limits of use

Not recommended to use for real-time PCR with fluorescently labeled probes. **BioMaster HS-qPCR (2×)** or **BioMaster UDG 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 UDG HS-qPCR SYBR Blue (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. Gently vortex and remove droplets by centrifugation.
4. Perform PCR using temperature conditions recommended below:

Step	Temperature, °C	Incubation time	Number of cycles
Anti-contamination treatment	50	2 min	1
Preliminary denaturation	95	5-7 min	1
Denaturation	95	15 sec	30 - 50
Annealing	50 - 68	10-30 sec	
Elongation	58 - 72	30-60 sec	
Melting curve (recommended)	65 - 95		1

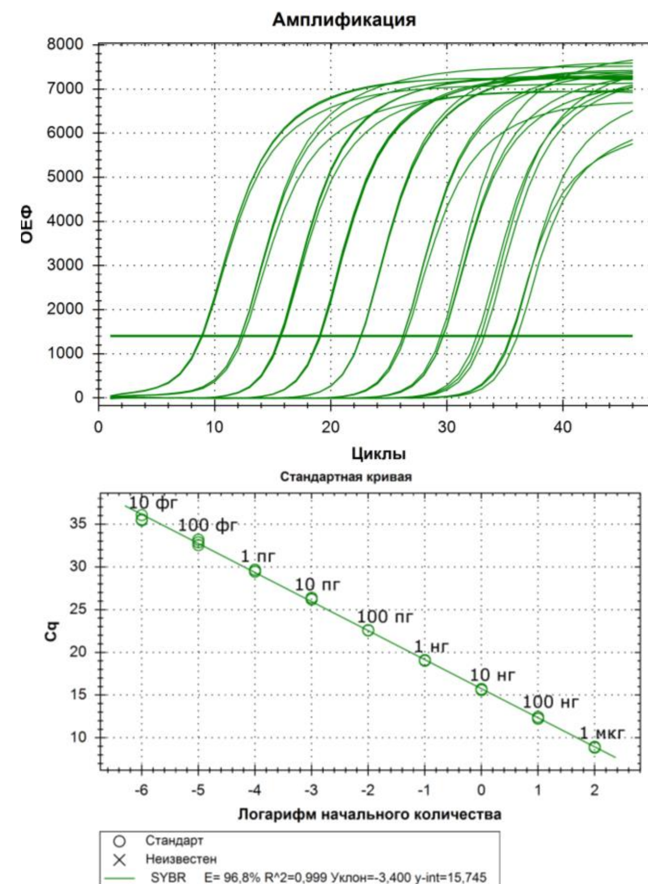
5. PCR results are displayed as amplification curves.

Note: Monitoring of real-time PCR can be performed at 72 °C in case if nonspecific products (primer-dimers) are absent. In case if non-specific products are generated, the Tm_1 of which is 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 UDG HS-qPCR SYBR Blue (2×)



*Amplification of the 18S mRNA gene fragment in 10-fold serial dilutions of cDNA (10 fg - 1 µg). Amplicon length: 120 bp. The reaction was performed using **CFX96 Touch** thermal cycler (Bio-Rad). Amplification curves and standard curve show linearity range.*

Recommendations for preventing PCR contamination.

More than 10 million copies of DNA template are produced during PCR. Thus, it is necessary to monitor the possibility of contamination of the reaction solution with other templates and amplicons that may be present in the laboratory. Here are the general recommendations for reducing the risk of contamination:

- Preparation of DNA samples and PCR solutions, amplification and analysis of PCR products must be carried out in different areas.

- Prepare PCR solutions in PCR UV-equipped laminar flow cabinets.
- Use different pairs of gloves for DNA purification and preparation of PCR solutions.
- Use reagents specifically designed for PCR. Use pipette tips equipped with an aerosol filter to prepare DNA samples and mix PCR components.
- Always prepare a template-free PCR sample (negative control) in order to detect/confirm the absence of contamination.

Recommendations for primer selection

For primer selection, use such highly-recommended software as Primer3 http://biotools.umassmed.edu/bioapps/primer3_www.cgi or Oligo http://www.oligo.net/. Follow the main principles of PCR primer design:

- Primer length should be within the range of 18 – 30 bp.
- Melting temperature (Tm): 58 – 65 °C. Difference in Tm of the two primers should not exceed 3 °C.
- Recommended length of real-time PCR products is 50 – 200 bp.
- Optimal GC composition of primers is within the range of 40 to 60%. Ideally, G and C nucleotides should be distributed evenly along the primer.
- Avoid the presence of more than 3 G/C nucleotides in a row at the 3'-end of the primer in order to decrease the risk of non-specific annealing.
- If possible, there should be G or C nucleotide at the 3'-end of the primer.
- Do not use primers with self-complementary and complementary regions to avoid formation of hairpins and primer dimers.
- Check for the presence of undesirable complementary sites between primers and DNA template.
- Always ckeck your primers using BLAST.

Reaction solution components

DNA template

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

Primers

Recommended concentrations of PCR primers are in the range of 0.1 – 0.6 µM. Excessive primer concentrations increase the chance of non-specific template binding and generation of alternative PCR products.

For degenerated primers and primers used for PCR of long fragments, we recommend using higher concentrations: 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 deoxyribonucleotides at 2:1 ratio. Therefore, additional optimization of Mg^{2+} concentration may be necessary in case if dNTP concentration is altered in the reaction solution. The recommended concentration of Mg^{2+} is 1-5 mM. In case if Mg^{2+} concentration is rather low, the yield of PCR product will be reduced. On the other hand, generation of non-specific products and decrease in PCR specificity can take place at high Mg^{2+} concentration. In case if DNA sample contains EDTA or other substances, chelating metals, concentration of Mg^{2+} in the reaction solution should be increased proportionally (EDTA binding occurs at the ratio of 1:1).

Characteristics of amplification steps

Initial DNA denaturation and enzyme activation

It is very important to achieve complete denaturation of the 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 5-minute denaturation at 95 °C will be enough.

Denaturation

Standard denaturation time per cycle for real-time PCR is 15 - 30 sec at 95 °C. In case of using GC-rich DNA templates, this step can be increased to 3-4 minutes.

Primer annealing

Primer annealing temperature should be 5 °C lower than the corresponding melting temperature (Tm). The general annealing time is 30 sec. In case if non-specific PCR products are formed, annealing temperature should be optimized using step-by-step increase of 1-2 °C.

Elongation

Taq DNA polymerase activity optimum is within the range of 70-75 °C. The rate of synthesis by *Taq* DNA polymerase varies 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 average rate of 1 min/kb.

Number of cycles

In case of less than 10 copies of DNA template per reaction, efficient amplification requires not less than 40 cycles. A total of 25 – 35 cycles is enough for a larger amount of template.

Final elongation

Additional 5 – 15-minute incubation of the reaction solution at 72 °C is recommended after the last cycle in order to complete synthesis of the PCR products. If the PCR product is to be further cloned into a TA vector, final elongation should be extended to 30 min in order to achieve maximal efficiency of 3'-dA end formation in PCR products.

Exodeoxyribonuclease activity

DNA showed stability 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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