Product information

BioMaster UDG HS-qPCR (2×)

Product description

BioMaster UDG HS-qPCR (2×) contains 2× ***BioMaster UDG HS-qPCR (2×)*** reaction mix and sterile water. ***BioMaster UDG HS-qPCR (2×)*** reaction mix is developed for quantitative real-time PCR with fluorescently labeled probes. ***BioMaster UDG HS-qPCR (2×)*** includes all of the components necessary for PCR (except for DNA template, primers and probe):

- highly-processive recombinant HS-*Taq* DNA polymerase;
- N-uracil-DNA glycosylase;
- deoxynucleoside triphosphate mixture;
- PCR buffer;
- Mg^{2+}.

The mix is optimized for efficient and reproducible real-time hot-start PCR of genomic, plasmid and viral DNA samples. The reaction mix contains additional components increasing the half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. ***BioMaster UDG HS-qPCR (2×)*** reaction mix does not contain substances affecting primer annealing temperature and characteristics of template melting. 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 and requires preheating at 95 °C for 5 min. Use of the mix helps saving experimental time and minimizes contamination risk due to reduced number of pipetting steps.

Product composition

Cat. #	BioMaster UDG HS-qPCR (2×)	Water	Number of reactions (50 µl each)
MH021-200	4 × 1.25 ml	4 × 1.25 ml	200
MH021-1020	17 × 1.5 ml	3 × 1.8 ml	1020

BioMaster UDG HS-qPCR (2×) includes:

100 mM Tris-HCl (pH 8.5 at 25 °C) 100 mM KCl, deoxynucleoside triphosphate mix (including dUTP), 10 mM $MgCl_2$, 0.1 U/µl HS-*Taq* DNA polymerase, N-uracil-DNA glycosylase, 0.025% Tween 20, stabilizers of HS-*Taq* DNA polymerase.

Applications:

- real-time hot-start PCR with fluorescently labeled probes;
- Conventional PCR;
- High-throughput PCR;
- Multiplex 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.

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 (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 and sensitivity
- HS-*Taq* DNA polymerase activation requires not more than 5-minute heating
- High selectivity and reaction yield
- 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)
- Minimized efforts

Limits of use

Not recommended to use for real-time PCR with intercalating dyes. **BioMaster HS-qPCR SYBR Blue (2×)** or **BioMaster UDG HS-qPCR SYBR Blue (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 (2×)	25	1×
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
Probe	variable	0.1 – 300 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	

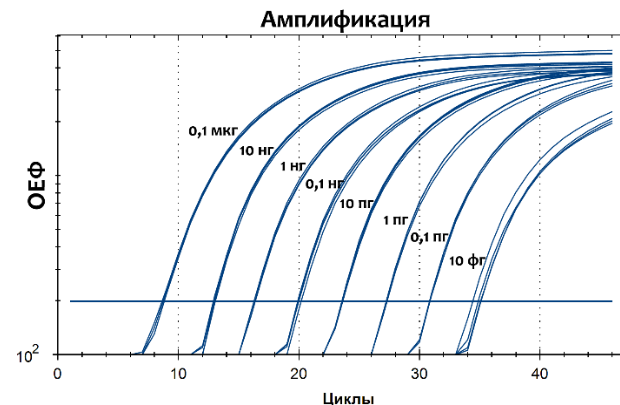
Or:

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/elongation	50 - 68	1 min	

5. PCR results are displayed as amplification curves.

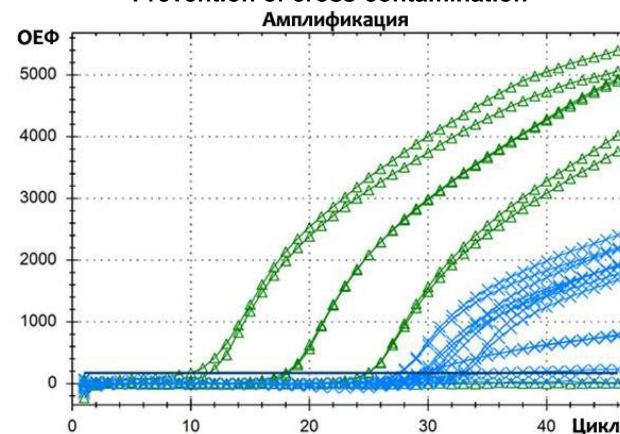
Storage and transportation: at -20 °C; not more than 50 thawing-freezing cycles.
Storage terms: 1 year (under proper storage and transportation conditions).

Linearity range of BioMaster UDG HS-qPCR (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).*

Prevention of cross-contamination



Amplification of serial dilutions (10^3 to 10^7-fold) of the reaction solution after PCR in the presence of dUTP. Green lines: BioMaster HS-qPCR (2×); blue lines: **BioMaster UDG HS-qPCR (2×)**.

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 laminar flow cabinets equipped with a UV lamp.
- 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 and probe design, we recommend using Oligo software http://www.oligo.net/ and its analogs. When selecting oligonucleotides, follow the basic principles:

Primers

- Primer length:18-22 bp.
- Difference in melting temperatures (Tm) of the two primers should not exceed 2 °C.
- Tm of the primers for TaqMan PCR should be ≥ 60 °C.
- GC composition of primers should be within the range of 40 to 60%.
- Product length: 70 – 150 bp.
- Minimize formation of secondary structures. Avoid them, if possible.
- Always ckeck your primers using BLAST.

Probes

- Probe length 22-26 bp.
- Melting temperature: 68-70 °C.
- There should be minimum of the same nucleotides in a row (especially G: not more than 4 G in a row).
- Chose the DNA strand that has more C than G nucleotides in it.
- There should be no G at 5'-end.
- Avoid self-complementarity and formation of dimers between probe and primers.

DNA samples

- Purity and integrity of DNA is extremely important for successful PCR. For DNA isolation, apply conventional methods that allow further amplification of the sample.
- Avoid using PCR inhibitors (phenol, hemin, etc.) when working with the samples. In case of using gel purification, minimize UV exposure for prevention of pyrimidine dimer formation.
- Prepare reaction solution in a clean area, use pipette tips with integrated filter in order to reduce contamination risk.
- Optimum amount of DNA per reaction depends on the sample type and its purity: phage lambda DNA ~0.1 ng; *E.coli* DNA ~10 ng; human DNA ~10 – 50 ng.

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 denaturation at 95 °C for 5 min will be enough.

Denaturation

Standard denaturation time per cycle for real-time PCR is 15 - 30 sec at 95 °C.

Primer annealing and elongation

For TaqMan real-time PCR, annealing and elongation stages are usually combined into one step at 58-60 ºC for 60 sec.

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.

Exodeoxyribonuclease activity

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

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