



Limited liability company

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BioMaster UDG HS-qPCR SYBR Blue (2x)

Cat. number MHC031-400, MHC031-2040

Product description:

BioMaster UDG HS-qPCR SYBR Blue (2x) kit contains 2x **BioMaster UDG HS-qPCR SYBR Blue (2x)** reaction mix, 50 mM MgCl₂ solution and sterile water. **BioMaster UDG HS-qPCR SYBR Blue (2x)** is developed for quantitative real-time PCR with fluorescent dye SYBR Green. **BioMaster UDG HS-qPCR SYBR Blue (2x)** contains all of the components necessary for PCR (except for DNA template and primers):

- highly-processive recombinant HS- *Taq* DNA polymerase;
- uracil-DNA glycosylase;
- deoxynucleoside triphosphate mix;
- PCR buffer;
- Mg²⁺ (3 mM);
- SYBR Green I;
- inert dye.

The mix is optimized for consistent and efficient real-time hot-start PCR of genomic, plasmid and viral DNA samples. The mix is supplemented with additives that increase half-life and processivity of HS- *Taq* DNA polymerase by enhancing its stability during PCR.

BioMaster UDG HS-qPCR SYBR Blue (2x) contains substances that affect primer annealing temperature and characteristics of template melting, which allows to enhance the specificity of PCR and use templates with complicated spatial structure.

The presence of uracil-DNA glycosylase and dUTP (proportional to TTP) provides reliable protection against reamplification of carryover PCR products between reactions (cross-contamination). DNA polymerase included in the kit is inactive at room temperature, and its activation requires preheating of the reaction solution at 95 °C for 5 min.

Blue dye of **BioMaster UDG HS-qPCR SYBR Blue (2x)** solution provided by the inert dye enables control over pipetting when using multi-well plates. The presented kit saves time and minimizes contamination risk due to reduced number of pipetting steps. Low Mg²⁺ concentration in master mix and additional solution of 50 mM MgCl₂ allow easy optimization of reaction conditions for individual primer/template pair.

Product composition

Cat. #	BioMaster UDG HS-qPCR SYBR Blue (2x)	50 mM MgCl ₂	Water	Number of reactions (25 µl)
MHC031-400	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	400
MHC031-2040	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	2040

BioMaster UDG HS-qPCR SYBR Blue (2x) contains:

100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, deoxynucleoside triphosphate mix (including dUTP), 3 mM MgCl₂, 0.12 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.

Area of application:

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

HS-Taq DNA Polymerase features

Recombinant *HS-Taq* DNA polymerase has 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* DNA polymerase depends on the complexity of DNA template and is approximately 2 kbp/min. Recombinant *HS-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 choice for detection and quantitative assessment of PCR products during real-time PCR without a need for 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 with all of the real-time PCR platforms existing to date.

Inert dye

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

Reaction mix features

- The mix is inactive at room temperature due to the "hot-start" technology and activated after incubation at 95 °C for 5 min;
- The presence of dUTP provides incorporation of uridine in every synthesized DNA chain; uracil-DNA glycosylase is capable of eliminating uracil from single- and double-stranded DNA molecules;
- Optimized for specific performance of *HS-Taq* DNA polymerase, long-term storage (storage of **BioMaster UDG HS-qPCR SYBR Blue (2x)** for 7 days at room temperature does not reduce PCR efficiency), multiple freezing-thawing cycles.

Benefits of use

- The enzyme with hot start capability increases reaction specificity and sensitivity;
- Activation of *HS-Taq* DNA polymerase requires not more than 5 min heating;
- The mix is colored for easy pipetting;
- Reduced preparation time;

- Protection against cross-contamination;
- 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. For such purposes, **BioMaster HS-qPCR (2x)** or **BioMaster UDG HS-qPCR (2x)** should be used.

Amplification protocol

1. DeThaw the reaction mixture and vortex thoroughly.
2. Add the following components into thin-wall PCR tubes considering the final volume of a reaction mixture equal to 25 μ L:

Component	Volume	Final concentration
BioMaster UDG HS-qPCR SYBR Blue (2x)	12,5	1x
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
DNA template	variable	1 pg – 1 μ g
Sterile water	up to 25 μ L	

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 min	1
Denaturation	95	5-15 sec	
Annealing	50 – 68	5-15 sec	30-50
Elongation	58 – 72	10-30 sec	
Melting curve (recommended)	65 – 95		1

5. PCR result is displayed as amplification curve.

Note: Real-time monitoring of PCR can be conducted at 72 °C in case if non-specific products (primer-dimers) are absent. If non-specific products with Tm1 lower than Tm2 of the target protein are formed, then reaction monitoring should be conducted at the temperature between Tm1 and Tm2.

Storage conditions: in a place protected from light at +25 °C – 7 days; at +4 °C – 4 months; at -20 °C – 18 months; not more than 50 thawing-freezing cycles.

Transportation: Transport in thermocontainers with cooling elements; the ambient temperature increment to the room temperature during the transportation up to 7 days is allowed.