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BioMaster HS-qPCR SYBR Blue (2×)

Cat. number MHC030-400, MHC030-2040

Product description:

BioMaster HS-qPCR SYBR Blue (2×) kit contains 2× **BioMaster HS-qPCR SYBR Blue** reaction mix, 50 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. **BioMaster HS-qPCR SYBR Blue (2×)** includes all components necessary for PCR (except for DNA template and primers):

- highly processive recombinant HS-*Taq* DNA polymerase;
- deoxynucleoside triphosphate mix;
- PCR buffer;
- Mg^{2+};
- SYBR Green I;
- inert dye.

The mix is optimized for conducting consistent and efficient real-time hot start PCR of genomic, plasmid and viral DNA samples. The solution includes substances increasing half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. ***BioMaster HS-qPCR SYBR Blue (2×)*** contains 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.

The 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 Mg2+ concentration in the reaction solution and additional tube with 50 mM MgCl2 included in the kit allows optimization of reaction conditions for individual primer pairs.

Product composition

Cat. #	BioMaster HS-qPCR SYBR Blue (2×)	50 мМ $MgCl_2$	Water	Number of reactions (25 µl)
MHC030-400	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	400
MHC030-2040	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	2040

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

100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, 0.4 mM of 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, 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 1 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 way 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 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 the blue dye is 615 nm.

Reaction mix features

- The mix is optimized for real-time PCR;
- The mix contains substances increasing storage time (storage of BioMaster HS-qPCR SYBR Blue (2×) for a month 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;
- 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. Thaw the reaction mixture and vortex thoroughly.
2. Put thin-wall PCR tubes on ice and add the following components considering the final volume of a reaction mixture equal to 25 µl:

Component	Volume	Final concentration
BioMaster HS-qPCR SYBR Blue (2×)	12,5	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 25 µl	

3. Carefully vortex and remove droplets by centrifugation.
4. Perform PCR using recommended conditions listed below:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5-15 sec	25-50
Annealing	50 - 68	5-15 sec	
Elongation	58 - 72	10-30 sec	
Melting curve (recommended)	65 - 95		1

5. PCR result is displayed as amplification curve.

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

Storage conditions: in a place protected from light: at +25 ° C – 7 days; at +4 ° C – 4 months; at -20 ° C – 18 months; no 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 10 days is allowed.