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BioMaster HS-qPCR Hi-ROX SYBR (2×)

Cat. number MHR030-400, MHR030-2040

Product description:

BioMaster HS-qPCR Hi-ROX SYBR (2×) kit contains 2× **BioMaster HS-qPCR Hi-ROX SYBR (2×)** reaction mix, 50 mM $MgCl_2$ solution and sterile water. **BioMaster HS-qPCR Hi-ROX SYBR (2×)** is developed for quantitative real-time PCR with fluorescent dye SYBR Green I on PCR platforms that use fluorescent dye ROX as a reference. **BioMaster HS-qPCR Hi-ROX SYBR (2×)** contains all of the components necessary for PCR (except for DNA template and primers):

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

The mix is optimized for conducting consistent and efficient real-time hot-start PCR of genomic, plasmid and viral DNA samples. The components of **BioMaster HS-qPCR Hi-ROX SYBR (2×)** 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 kit is inactive at room temperature; its activation requires preheating of the reaction solution at 95 °C for 5 min.

Blue hue of **BioMaster HS-qPCR Hi-ROX SYBR (2×)** 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 Mg^{2+} concentration in master mix and additional solution of 50 mM $MgCl_2$ allow easy optimization of reaction conditions for individual primer/template pair.

The master mix is ideally suitable for PCR platforms that use ROX passive dye as a reference guide: Life Technologies (ABI) 7000, 7300, 7700, 7900, 7900HT, StepOne Plus.

Product composition

Cat. #	BioMaster HS-qPCR Hi-ROX SYBR (2×)	50 mM $MgCl_2$	Water	Number of reactions (25 µl)
MHR030-400	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	400
MHR030-2040	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	2040

BioMaster HS-qPCR Hi-ROX SYBR (2×) contains:
100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, 0.4 mM of each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 0.12 u/µl HS-*Taq* DNA polymerase, 0.025% Tween 20, stabilizers of *HS-Taq* DNA polymerase, SYBR Green I, 0.9 µM fluorescent dye ROX, 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.

Passive reference dye ROX

The mix includes passive fluorescent dye ROX, which serves as the inner reference for SYBR Green I signal normalization when using PCR platforms supporting such function (Applied Biosystems). ROX allows adjusting variations between tubes (wells) that occur due to the pipetting errors and fluctuation in fluorescence. The presence of ROX does not affect the course of PCR and shift in fluorescence signal in case if the mix is used with other PCR platforms. However, it should be taken into account that the presence of ROX fluorophore restricts its use for oligonucleotide probes, as well as for other dyes that share similar spectral characteristics (Em ~ 621 nm).

Inert dye

The inert dye included in BioMaster HS-qPCR Hi-ROX SYBR (2×) 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.;
- Allows normalization to ROX reference dye;

- Optimized for specific performance of *HS-Taq* DNA polymerase, long-term storage (storage of **BioMaster HS-qPCR Hi-ROX SYBR (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;
- Activation of HS-*Taq* DNA polymerase requires not more than 5 min heating;
- Enhanced selectivity and reaction yield;
- The mix is colored for easy pipetting;
- Reduced preparation time;
- Low contamination risk when mixing PCR components;
- Provides data normalization;
- 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 Hi-ROX (2×)** or **BioMaster UDG HS-qPCR Hi-ROX (2×)** should be used for such purposes.

Amplification protocol

1. Thaw 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 µl:

Component	Volume	Final concentration
BioMaster HS-qPCR Hi-ROX SYBR (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	

2. Carefully vortex and remove droplets by brief centrifugation.
4. Perform PCR, using temperature conditions recommended below:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5-15 sec	30-50
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: 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 10 days is allowed.