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BioMaster HS-qPCR Lo-ROX (2×)

Cat. number MHR021-400, MHR021-2040

Product description:
BioMaster HS-qPCR Lo-ROX (2×) kit contains ready-to-use **BioMaster HS-qPCR Lo-ROX (2×)** master mix and sterile water. **BioMaster HS-qPCR Lo-ROX (2×)** is developed for quantitative real-time PCR with fluorescent probes on PCR platforms that use ROX as a reference dye. **BioMaster HS-qPCR Lo-ROX (2×)** includes all the components necessary for PCR (excluding DNA template, primers and probe):

- highly processive recombinant HS-Taq DNA polymerase;
- deoxynucleoside triphosphate mix;
- PCR buffer;
- Mg^{2+} (5 mM);
- ROX 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 HS-qPCR Lo-ROX (2×)** does not contain substances affecting primer annealing temperature and characteristics of template melting.

DNA polymerase included in the **BioMaster HS-qPCR Lo-ROX (2×)** is inactive at room temperature, and its activation requires preheating of the reaction solution at 95 °C for 5 min.

The master mix is ideally suitable for PCR platforms that use ROX passive dye as a reference guide: Life Technologies (ABI) 7500, 7500 Fast, ViiA 7, QuantStudio 12K; Stratagene Mx4000, Mx3005P, Mx3000P etc. Use of the kit saves time and minimizes contamination risk due to reduced number of pipetting steps.

Product composition

Cat. #	BioMaster HS-qPCR Lo-ROX (2×)	Water	Number of reactions (25 µl)
MHR021-400	4 × 1.25 ml	4 × 1.25 ml	400
MHR021-2040	17 × 1.5 ml	3 × 1.8 ml	2040

BioMaster HS-qPCR Lo-ROX (2×) contains:
100 mM Tris-HCl (pH 8.5 at 25 °C), 100 mM KCl, 0.4 mM each deoxynucleoside triphosphate, 10 mM $MgCl_2$, 0.1 U/µL HS-*Taq* DNA polymerase, 0.025% Tween 20, stabilizers of *HS-Taq* DNA polymerase, 60 nM ROX fluorescent dye.

Limits of use:
Not recommended to use for real-time PCR with intercalating dyes. **BioMaster HS-qPCR Lo-ROX SYBR (2×)** or **BioMaster UDG HS-qPCR Lo-ROX SYBR (2×)** should be used for such purposes.

Area of application:
- Real-time hot start PCR with fluorescently labeled probes and ROX as a reference dye;
- Conventional PCR;
- High-throughput PCR;
- Multiplex PCR;
- Genotyping.

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 extension rate of *Taq* DNA polymerase depends on the complexity of DNA template and is approximately 1 kbp/min. Recombinant form of the enzyme is ideal for both conventional and real-time PCR.

Passive fluorescent ROX dye
The mix includes passive fluorescent ROX dye, which serves as the inner reference for signal normalization of dyes comprising oligonucleotide probes when using PCR platforms with such function (Applied Biosystems). ROX allows adjustment of variations between tubes (wells) that occur due to the pipetting errors and fluorescence fluctuation. 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).

Product features:
- The mix is optimized for real-time hot-start PCR with fluorescently labeled probes;
- Allows normalization to ROX reference dye;
- Prevents re-amplification of extraneous PCR products;
- The mix contains substances that increase its storage terms (the storage of **BioMaster HS-qPCR Lo-ROX (2×)** at room temperature for 7 days does not reduce PCR efficiency) and allow multiple thawing-freezing cycles.

Benefits of use:
- The enzyme with hot start capability enhances reaction specificity;
- Activation of HS-*Taq* DNA polymerase requires not more than 5 min heating;
- High selectivity and reaction yield;
- Reduced preparation time;
- Low chance of contamination during preparation of PCR solution;
- Possibility of data normalization;
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components in a series of experiments);

- Minimized efforts.

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 Lo-ROX (2×)	12,5	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 25 µL	

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

Three-step protocol:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5 - 15 sec	30-50
Annealing	50 - 68	10 - 30 sec	
Elongation	72	5-30 sec	

Or:

Two-step protocol:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5 - 15 sec	30-50
Annealing/elongation	50 - 68	30-60 sec	

5. PCR result is displayed as amplification curve.

Storage conditions: in a place protected from light at +4 ° C - 3 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.