

## Product information

### BioMaster HS-qPCR Lo-ROX SYBR (2×)

#### Product description

**BioMaster HS-qPCR Lo-ROX SYBR (2×)** kit includes 2× **BioMaster HS-qPCR Lo-ROX SYBR** reaction mix, 50 mM MgCl<sub>2</sub> solution and sterile water. **BioMaster HS-qPCR Lo-ROX SYBR (2×)** reaction mix is developed for quantitative real-time PCR with SYBR Green I fluorescent dye on PCR platforms that utilize ROX as a reference dye. The master mix contains all of the components necessary for PCR (except for DNA template and primers):

- highly-processive recombinant HS-*Taq* DNA polymerase;
- deoxynucleoside triphosphate mixture;
- PCR buffer;
- Mg<sup>2+</sup> (3 mM);
- SYBR Green I;
- ROX fluorescent dye;
- Inert dye.

The mix is optimized for efficient and reproducible real-time hot-start PCR of genomic, plasmid and viral DNA samples. **BioMaster HS-qPCR Lo-ROX SYBR (2×)** reaction mix contains substances that influence primer annealing temperature and characteristics of template melting, which enhance PCR specificity and allow usage of templates with complicated spatial structure.

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 due to the presence of 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 Mg<sup>2+</sup> concentration in the reaction mix and additional tube with 50 mM MgCl<sub>2</sub> solution included in the kit allow optimizing PCR conditions for individual primer-template system.

The master mix is ideally suitable for PCR platforms that use ROX passive dye as a reference guide, such as Life Technologies (ABI) 7500, 7500 Fast, ViiA 7, QuantStudio 12K; Stratagene Mx4000, Mx3005P, and Mx3000P.

#### Product composition

Cat. #	BioMaster HS-qPCR Lo-ROX SYBR (2×)	50 mM MgCl <sub>2</sub>	Water	Number of reactions (50 µl)
MHR031-200	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	200
MHR031-1020	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	1020

#### **BioMaster HS-qPCR Lo-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<sub>2</sub>, 0.12 U/µl HS-*Taq* DNA polymerase, 0,025% Tween 20, stabilizers of HS-*Taq* DNA polymerase, SYBR Green I, 60 nM ROX fluorescent dye, and inert dye.

#### Applications:

- Real-time hot-start PCR with intercalating dye SYBR Green I;
- Conventional PCR;
- High-throughput 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.

#### **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 of using specific fluorescent probes. During amplification, SYBR Green I dye intercalates into the minor groove of DNA products and emits stronger fluorescent signal than the unbound dye. Absorption and emission maxima of SYBR Green I are 494 nm and 521 nm, respectively, which enables using it for every real-time PCR platform existing to date.

#### **ROX passive fluorescent dye**

The mix contains passive fluorescent dye ROX, which serves as the inner standard for normalization of the signal of dyes comprising oligonucleotide probes on a series of PCR platforms (Applied Biosystems). ROX allows correction of fluctuations between the tubes (wells), which are due to the pipetting errors and fluorescence fluctuation. ROX does not affect PCR and fluorescence signal in case if the mix is used for other PCR platforms. However, it should be taken into account that the presence of ROX in the master mix limits its use only for oligonucleotide probes, as well as other dyes that share similar spectral characteristics (Em ~ 621 nm).

#### **Inert dye**

The inert dye comprising **BioMaster HS-qPCR Lo-ROX SYBR (2×)** does not reduce PCR efficiency and facilitates multi-well plate pipetting. Absorption maximum of the blue dye is 615 nm.

#### **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;
- Allows normalization using ROX fluorescent dye as a reference;
- The mix is optimized for specific performance of HS-*Taq* DNA polymerase, long-term storage (the storage of **BioMaster HS-qPCR Lo-ROX SYBR (2×)** at room temperature for a month does not affect PCR efficiency), multiple thawing-freezing cycles.

#### **Benefits of use**

- Hot-start enzyme increases reaction specificity, sensitivity and reaction yield
- HS-*Taq* DNA polymerase activation requires not more than 5-minute heating
- The mix is colored for easy pipetting
- Reduced preparation time
- Low contamination risk during preparation of PCR solution
- Allows data normalization
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components from experiment to experiment)

### Limits of use

Not recommended to use for real-time PCR with fluorescently labeled probes. **BioMaster HS-qPCR Lo-ROX (2x)** and **BioMaster UDG HS-qPCR Lo-ROX (2x)** should be used for such purposes.

### Amplification protocol

- Defrost the reaction mixture and stir thoroughly.
- Add the following components into the thin-wall PCR tubes considering the final volume of a reaction mixture equal to 50  $\mu$ l:

Component	Volume	Final concentration
<b>BioMaster HS-qPCR Lo-ROX SYBR (2x)</b>	25	1x
<b>Forward primer</b>	variable	0.1 – 600 nM
<b>Reverse primer</b>	variable	0.1 – 600 nM
<b>DNA template</b>	variable	1 pg – 1 $\mu$ g
<b>Sterile water</b>	up to 50 $\mu$ l	

- Gently vortex and remove droplets by centrifugation.
- Conduct PCR using temperature conditions recommended below:

Step	Temperature, °C	Incubation time	Number of cycles
<b>Preliminary denaturation</b>	95	5-7 min	1
<b>Denaturation</b>	95	15 sec	30 - 50
<b>Annealing</b>	50 - 68	10-30 sec	
<b>Elongation</b>	58 - 72	30-60 sec	
<b>Melting curve (recommended)</b>	65 - 95		1

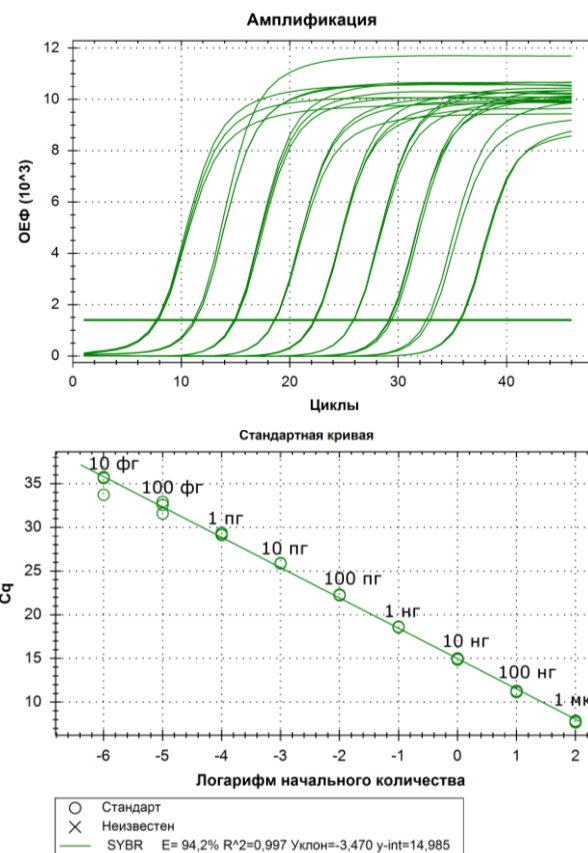
- PCR results are displayed as amplification curves.

*Note:* Monitoring of real-time PCR can be performed at 72 °C in case if nonspecific products (primer-dimers) are absent. In case if non-specific products are generated, the  $T_{m1}$  of which is lower than  $T_{m2}$  of the main product, monitoring should be performed at temperatures between  $T_{m1}$  and  $T_{m2}$ .

**Storage and transportation:** at -20 °C; not more than 50 thawing-freezing cycles.

**Storage terms:** 1 year (under proper storage and transportation conditions).

### Amplification curves and standard curve obtained during real-time PCR using BioMaster HS-qPCR Lo-ROX SYBR (2x)



Amplification of the 18S mRNA gene fragment in 10-fold serial dilutions of cDNA (10 fg - 1  $\mu$ g). Amplicon length: 120 bp. The reaction was performed using **CFX96 Touch** thermal cycler (Bio-Rad). Amplification curves and standard curve show linearity range.

### 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 UV-equipped laminar flow cabinets.
- 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 selection, use such highly-recommended software as Primer3 [http://biotools.umassmed.edu/bioapps/primer3\\_www.cgi](http://biotools.umassmed.edu/bioapps/primer3_www.cgi) or Oligo <http://www.oligo.net/>. Follow the main principles of PCR primer design:

- Primer length should be within the range of 18 – 30 bp.
- Melting temperature (T<sub>m</sub>): 58 – 65 °C. Difference in T<sub>m</sub> of the two primers should not exceed 3 °C.
- Recommended length of real-time PCR products is 50 – 200 bp.
- Optimal GC composition of primers is within the range of 40 to 60%. Ideally, G and C nucleotides should be distributed evenly along the primer.
- Avoid the presence of more than 3 G/C nucleotides in a row at the 3'-end of the primer in order to decrease the risk of non-specific annealing.
- If possible, there should be G or C nucleotide at the 3'-end of the primer.
- Do not use primers with self-complementary and complementary regions to avoid formation of hairpins and primer dimers.
- Check for the presence of undesirable complementary sites between primers and DNA template.
- Always check your primers using BLAST.

### Reaction solution components

#### DNA template

Optimal amount of DNA template per 50 µl reaction solution is 0.01 – 1 ng for plasmid or phage DNA and 0.1 – 1 µg for genomic DNA. Higher amounts of template increase the risk of non-specific product formation, low amounts of template reduce amplification accuracy. All conventional techniques of DNA purification can be applied for preparation of the analyzed sample. It should be taken into account that trace amounts of certain agents used for DNA isolation and purification, such as phenol, EDTA, and proteinase K, can inhibit DNA polymerase. Precipitation and repeated washing with 70% ethanol solution usually remove trace contaminants from the DNA sample.

#### Primers

Recommended concentrations of PCR primers are in the range of 0.1 – 0.6 µM. Excessive primer

concentrations increase the chance of non-specific template binding and generation of alternative PCR products.

For degenerated primers and primers used for PCR of long fragments, we recommend using higher concentrations: 0.3 – 1 µM.

#### Mg<sup>2+</sup> concentration

Change in the concentration of Mg<sup>2+</sup> ions can have a significant impact on PCR efficiency and specificity. These ions are necessary for the performance of *Taq* DNA polymerase. They also bind deoxyribonucleotides at 2:1 ratio. Therefore, additional optimization of Mg<sup>2+</sup> concentration may be necessary in case if dNTP concentration is altered in the reaction solution. The recommended concentration of Mg<sup>2+</sup> is 1-5 mM. In case if Mg<sup>2+</sup> concentration is rather low, the yield of PCR product will be reduced. On the other hand, generation of non-specific products and decrease in PCR specificity can take place at high Mg<sup>2+</sup> concentration. In case if DNA sample contains EDTA or other substances, chelating metals, concentration of Mg<sup>2+</sup> in the reaction solution should be increased proportionally (EDTA binding occurs at the ratio of 1:1).

### 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 1- to 3-minute denaturation at 95 °C will be enough.

#### Denaturation

Standard denaturation time per cycle for real-time PCR is 30 sec at 95 °C. In case of using GC-rich DNA templates, this step can be increased to 3-4 minutes.

#### Primer annealing

Primer annealing temperature should be 5 °C lower than the corresponding melting temperature (T<sub>m</sub>). The general annealing time is 30 sec. In case if non-specific PCR products are formed, annealing temperature should be optimized using step-by-step increase of 1-2 °C.

#### Elongation

*Taq* DNA polymerase activity optimum is within the range of 70-75 °C. The rate of synthesis by *Taq* DNA polymerase varies from 30 to 60 bp per second depending on template complexity. In the case of using long templates (>2 kbp), it is recommended to estimate elongation time based on the average rate of 1 min/kb.

#### 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 higher amount of template.

#### Final elongation

Additional 5 – 15-minute incubation of the reaction solution at 72 °C is recommended after the last cycle in order to complete synthesis of the PCR products. If the PCR product is to be



further cloned into a TA vector, final elongation should be extended to 30 min in order to achieve maximal efficiency of 3'-dA end formation in PCR products.

***Exodeoxyribonuclease activity***

DNA showed stability after incubation of 1 µg fragment of phage lambda DNA in the presence of 25 µl of **BioMaster HS-qPCR Lo-ROX SYBR (2x)** 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<sup>32</sup>]-labeled RNA fragment in the presence of 25 µl of **BioMaster HS-qPCR Lo-ROX SYBR (2x)** in 50 µl reaction solution at 37 °C for 4 h.

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