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BioMaster LR HS-PCR-Color (2×)

Cat. number MHC040-100, MHC040-400

Product description

BioMaster LR HS-PCR-Color (2×) contains 2× ***BioMaster LR HS-PCR-Color*** reaction mix, DMSO and sterile water. **BioMaster LR HS-PCR-Color (2×)** reaction mix is developed for amplification of long DNA fragments (0.2 to 30 kbp) with high fidelity, increased specificity and productivity. The master mix is ideally suitable for amplification of GC-rich (>65%) and complicated DNA regions. **BioMaster LR HS-PCR-Color (2×)** contains all of the components necessary for PCR (except for DNA template and primers):

- mix of polymerases (HS-*Taq* and *Pfu*),
- deoxynucleoside triphosphate mix,
- PCR buffer,
- Mg^{2+}.

BioMaster LR HS-PCR-Color (2×) contains a combination of two highly purified enzymes: highly-processive recombinant HS-*Taq* DNA polymerase and *Pfu* DNA polymerase with error-correcting activity. Such blend of polymerases is inactive at room temperature (hot-start variant), and their activation requires preheating at 95 °C for 5 min.

Represents a blend of polymerases enabled to enhance amplification fidelity and reliability several fold compared to PCR with *Taq* polymerase only. Synergic performance of the two enzymes allows generating PCR products up to 30 kbp in length. The products obtained using **BioMaster LR HS-PCR-Color (2×)** mostly contain 3′-dA ends and can be further used for cloning.

The buffer is optimized for efficient performance of both polymerases and provides maximal reaction yield. High density of the solution and the presence of tracing dyes allow direct gel loading.

The developed kit for PCR saves time and minimizes contamination risk due to reduced number of pipetting steps. Dyes included in the mix do not interfere with amplicon purification by most of the existing methods.

Product composition

Catalogue number	*BioMaster LR HS-PCR-Color (2×)*	Water	DMSO	Number of reactions (50 µl each)
MHC040-100	2 × 1.25 ml	2 × 1.25 ml	1× 0.2 ml	100
MHC040-400	6 × 1.67 ml	3 × 1.8 ml	1× 1 ml	400

BioMaster LR HS-PCR-Color (2×) contains:

1100 mM Tris-HCl (pH 8.9 at 25 °C), 100 mM KCl, 0.8 mM of each deoxynucleoside triphosphate, 4 mM $MgSO_4$, 0.1 U/µl polymerase mix, 0.2% Tween 20, stabilizers of DNA polymerases, and tracing dyes.

Area of application:

- Long-range PCR;
- Product generation for TA-cloning;
- Amplification of GC-rich and complicated templates.

Features of polymerase mix

The blend of DNA polymerases is specifically developed for efficient amplification of DNA fragments 0.2 to 30 kbp in length from various templates. The developed blend has 5′-3′ DNA-dependant polymerase, 5′-3′ exonuclease and 3′-5′ exonuclease (correcting) activities. The rate of DNA synthesis by *Taq* DNA polymerase depends on the complexity of DNA template and averages 1-2 kbp/min.

Reaction mix features

- Optimized for specific performance of HS-*Taq* and *Pfu* DNA polymerases;
- The composition allows long-term storage (the storage of **BioMaster LR HS-PCR-Color (2×)** at room temperature for 7 dayses not affect RT-PCR efficiency) and multiple thawing-freezing cycles (more than 50 times);
- Contains dyes that do not affect polymerase performance and components increasing solution density for easy gel loading.

Note: mobility of dyes in 0.5 – 1.5% agarose gel:

xylene cyanol	bromphenol blue	Orange G	tartrazine
10000 – 4000 bp	500-400 bp	<100 bp	<20 bp

Benefits of use:

- Generation of amplicons
  up to 30 kbp from viral DNA templates,
  up to 15 kbp from genomic DNA;
- Enhanced amplification fidelity compared to PCR with *Taq* DNA polymerase only;
- The enzyme with hot start capability increases specificity, sensitivity and reaction yield;
- Activation of the DNA polymerase mix requires not more than 5 min heating;
- Amplification of a wide range of DNA templates;
- Low contamination risk during preparation of PCR solution;
- Easy gel loading (no need in additional loading buffer due to high density of the master mix);
- PCR products can be further subjected to TA cloning due to the presence of deoxyadenosine overhangs in amplified DNA.

Amplification protocol

1. Thaw the reaction mixture, vortex carefully and thoroughly.
2. Put thin-wall PCR tubes on ice and add the following components considering the final volume of a reaction mixture equal to 50 µl:

Component	Volume	Final concentration
BioMaster LR HS-PCR-Color (2×)	25	1×
Forward primer	variable	0.1 – 800 nM
Reverse primer	variable	0.1 – 800 nM
DNA template	variable	1 – 500 ng
Sterile water	up to 50 µl	

Note: if necessary, add DMSO in the amount of 1 to 5% of the final volume of reaction solution. Shift in primer Tm should be taken into account while composing PCR program.

3. Carefully vortex and remove droplets by brief centrifugation.
Note: in case if a thermal cycler is not equipped with a heated lid, add a droplet (25-35 µL) of mineral oil in each tube.

4. A standard three-step program can be used for amplification of a 10 kbp DNA fragment. The following regimes are recommended for amplification of products more than 10 kbp in length (when choosing amplification program, please see the recommendations on its optimization).

Three-step program:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	92-94	2-4 min	1
Denaturation	92-94	10-20 sec	10
Annealing	50–68 (Tm-5)	30 sec	
Elongation	68	x min	
Denaturation	94	10-20 sec	15-20
Annealing	50–68 (Tm-5)	30 sec	
Elongation	68	x (+10 sec/cycle) min	
Final elongation	68	5 – 15 min	1

Tm – template/primer melting temperature, depends on primer structure. The following formula can be used for approximate estimation of Tm: Tm (°C) = 2 x (A+T) + 4 x (G+C).

Two-step program:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	92-94	2-4 min	1
Denaturation	92-94	10-20 sec	10
Annealing/elongation	68	x min	
Denaturation	94	10-20 sec	15-20
Annealing/elongation	68	x (+10 sec/cycle) min	
Final elongation	68	5 – 15 min	1

x – elongation time depends on the length of amplified sequence:

Amplicon length, kbp	3	6	10	15	20	30
Elongation time, min	2	4	8	13	16	22

5. After conducting PCR analyze amplification products by gel electrophoresis.
Note: for separation of reaction products by electrophoresis we recommend to use 1x TAE buffer with ethidium bromide.

Storage conditions: in a place protected from light at at +4 ° C - 3 months; at -20 ° C - 12 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.