Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

BioMaster LR HS-PCR (2×)

Cat. number MH040-100, MH040-400

Description:

BioMaster LR HS-PCR (2×) includes 2× **BioMaster LR HS-PCR** reaction mix, DMSO and sterile water. **BioMaster LR HS-PCR (2×)** is developed for amplification of long (0.2 to 30 kbp) DNA fragments with high fidelity, increased specificity and productivity. The mix is ideally suitable for amplification of GC-rich (>65%) and complicated DNA regions. **BioMaster LR HS-PCR (2×)** contains all 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 (2×) mix contains a combination of two highly purified enzymes: highly-processive 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.

The combination of two polymerases enabled to enhance amplification fidelity and reliability several fold compared to PCR performed 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 (2×)** mostly contain 3´-dA ends and thus can be further used for cloning.

The buffer is optimized for efficient performance of both polymerases and provides maximal reaction yield. Use of the kit saves time and minimizes contamination risk due to reduced number of pipetting steps.

Kit contains:

Catalogue number	*BioMaster LR HS-PCR (2×)*	Water	DMSO	6×loading buffer	Number of reactions (50 µl each)
MH040-100	2 × 1.25 ml	2 × 1.25 ml	1× 0.2 ml	1 × 0.5 ml	100
MH040-400	6 × 1.67 ml	2 × 1.8 ml	1× 1 ml	1 × 1 ml	400

BioMaster LR HS-PCR (2×) contains:

100 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.

Area of application:

- Long-range PCR;
- Product synthesis for TA-cloning;

- Amplification of GC-rich and complicated templates.

Features of polymerase mix

The mixture of DNA polymerases is specifically developed for efficient amplification of DNA fragments ranging from 0.2 to 30 kbp in length. The developed blend has 5′-3′ DNA-dependent 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 solution composition allows long-term storage (the storage of **BioMaster LR HS-PCR (2×)** at room temperature for 7 days does not affect the efficiency of RT-PCR) and multiple thawing-freezing cycles (more than 50 times);

Benefits of use:

- Generation of amplicons
  up to 30 kbp from viral DNA templates,
  up to 15 kbp from genomic DNA;
- Enhanced fidelity of amplification compared to PCR with *Taq* DNA polymerase only;
- Enzyme with hot start function enhances specificity, sensitivity and reaction yield;
- Activation of polymerase mix requires not more than 5 min heating;
- Reduced preparation time;
- Amplification of a wide range of DNA templates;
- Low contamination risk during preparation of PCR solution;
- 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, vortexcarefully and thoroughly.

2. Add the following components into thin-wall PCR tubes considering the final volume of a reaction mixture equal to 50 µl:

Component	Volume	Final concentration
BioMaster LR HS-PCR (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 the reaction mixture. A shift in primer Tm should be taken into account while composing PCR program.

3. Carefully vortex and remove droplets form the tube walls by 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 fragment. The following regimes are recommended for amplification of products more than 10 kbp in length (when choosing amplification program, please familiarize with 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.

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

Storage conditions: in a place, protected from the light, at +4 ° C - 3 months; at -20 ° C - 1 year; 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.