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BioMaster HS-*Taq* PCR Sp (2×)

Cat. number MH011-200, MH011-1020

Product description:

BioMaster HS-Taq PCR Sp (2×) have been developed for PCR with difficult templates that have a high degree of secondary structure or GC-rich segments. 2× **BioMaster HS-Taq PCR Sp (2×)** includes reaction mix, 50 mM $MgCl_2$, sterile water and 6× loading buffer. The mix has **BioMaster HS-Taq PCR Sp (2×)** contains all components (except for DNA template and primers) needed for a PCR reaction:

- highly processive recombinant HS-*Taq* DNA polymerase;
- deoxynucleoside triphosphate mix;
- PCR buffer;
- Mg^{2+}.

The mix is optimized for performing efficient and reproducible hot-start PCR with difficult templates that have a high degree of secondary structure or GC-rich segments. The mix contains additional components increasing the half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. **BioMaster HS-Taq PCR Sp (2×)** contains components, changing primers annealing temperature template melting parameters.

DNA polymerase included in the mix is inactive at room temperature and requires preheating at 95 °C for 5 min. Additional $MgCl_2$ solution allows easy optimization of the reaction mix for each individual primer/template system.

The presented kit from helps saving experimental time and minimizes contamination risk due to reduced number of pipetting steps.

Product composition

Cat. #	*BioMaster HS-Taq PCR Sp (2×)*	50 mM $MgCl_2$	Water	6× loading buffer	Number of reactions (50 µl)
MH011-200	4 × 1.25 ml	1 × 1 ml	4 × 1.25 ml	1 × 1 ml	200
MH011-1020	17 × 1.5 ml	1 × 1.8 ml	2 × 1.8 ml	2 × 1.8 ml	1020

BioMaster HS-Taq PCR Sp (2×) contains:
100 mM Tris-HCl (pH 8.5 at 25 °C) 100 mM KCl, 0.4 mM of each deoxynucleoside triphosphate, 4 mM $MgCl_2$, 0.06 U/µl *Taq* DNA polymerase, 0.2% Tween 20, DMSO, stabilizers of HS-*Taq* DNA polymerase.

Applications:

- Amplification of DNA matrixes with GC content over 66%
- Hot-start PCR
- Synthesis of PCR products for TA cloning
- RT-PCR

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 2 kbp/min. Recombinant HS-*Taq* DNA Polymerase is ideal for conventional PCR of templates up to 5 kbp.

Reaction mix features

- The reaction mix is optimized for specific performance of HS-*Taq* DNA polymerase, long-term storage (the storage of ***BioMaster HS-Taq PCR Sp (2×)*** at room temperature for 30 days does not affect PCR efficiency), multiple thawing-freezing cycles;
- The mix contains components increasing density of sample solution making it easier to work with
- The mix does not contain substances that interfere with optical monitoring of a reaction course and changes in sample fluorescence.

Benefits of use:

- Amplification of DNA matrixes with GC content over 66%;
- Hot-start enzyme increases specificity, sensitivity and reaction yield;
- HS-*Taq* DNA polymerase activation requires 5 min heating;
- Reduced preparation time;
- Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components from experiment to experiment);
- PCR products can be further subjected to TA cloning due to the presence of deoxyadenosine overhangs in amplified DNA.

Limits of use

- Not recommended to use for amplicons >5 kbp

Amplification protocol

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

Component	Volume	Final concentration
BioMaster HS-Taq PCR Sp (2×)	25	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 50 µl	

3. Gently vortex and remove droplets 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.
Note: prepared reaction mixture should be quickly placed into the pre-warmed to 95°C thermal cycler.

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	25-50
Annealing	50 – 68 (Tm-5)	5 - 20 sec	
Elongation	72	0.5-1 min/kbp	
Final elongation	72	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).
Note: the reaction mix contains compounds, lowering Tm by several degrees. It should be considered for choosing proper primers annealing temperature.

5. After performing PCR, analyze amplification products by gel electrophoresis. Samples are loaded on gel without additional loading buffer.

Note: we recommend using 1x TAE buffer with ethidium bromide for visualizing PCR products by gel electrophoresis.
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 light at +25 ° C - 7 days; at +4 ° C - 4 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.