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BioMaster HS-*Taq* PCR Kit

Cat. number KH017-500, KH017-2250

Product description:

HS-*Taq* PCR Kit contains recombinant HS-*Taq* DNA polymerase and the solutions containing all the essential components to perform conventional hot-start PCR, excluding DNA template and primers. The kit includes: HS-*Taq* DNA polymerase solution (5 U/µl), 5× PCR buffer, 50 mM $MgCl_2$, 50× dNTP mix and 6× loading buffer. All of the included reagents are high quality and optimized for PCR.

HS-*Taq* DNA polymerase is a recombinant *Taq* DNA polymerase inactivated by specific monoclonal antibodies. HS-*Taq* DNA polymerase is inactive at temperatures lower than 70 °C. It allows to avoid the formation of non-specific products and primer-dimers at low temperatures during sample preparation. Activation of HS-*Taq* DNA polymerase occurs during the first cycle of PCR amplification after short 5 min incubation at 95 °C. The recombinant form of the enzyme has 5′-3′ DNA-dependent DNA 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 2 kbp/min. Recombinant *Taq* DNA polymerase is ideal for conventional PCR of templates up to 5 kbp.

5× PCR buffer **(does not contain $MgCl_2$)** is optimized for efficient and reproducible PCR. The buffer components increase half-life and processivity of HS-*Taq* DNA polymerase by enhancing its stability during PCR. The buffer is chemically stable, inert and its composition does not affect optimal annealing temperature or the parameters of template melting. Additional solutions of 50 mM $MgCl_2$ and 50× dNTP mix included in the kit allow optimizing PCR conditions for individual primer pairs, while 6× loading buffer eases sample preparation for analysis and allows monitoring the progress of electrophoresis.

Product composition

Cat. #	HS-*Taq* DNA polymerase, 5 U/µl*	5× PCR buffer	50 mM $MgCl_2$	50× dNTP mix (10 mM each)	6× loading buffer	Activity units (U)
KH017-500	1 × 100 µl	2 × 1.5 ml	1 × 1 ml	2 × 200 µl	1 × 1.75 ml	500
KH017-2250	3 × 150 µl	8 × 1.5 ml	2 × 1 ml	4 × 400 µl	3 × 1.75 ml	2250

* One activity unit is the amount of the enzyme required to catalyze incorporation of 10 nmol of dNTP into an acid-insoluble product in 30 min at 74 °C. Reaction conditions: 50 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM NaCl, 10 mM $MgCl_2$, 200 mM dATP, 200 mM dCTP, 200 mM dGTP, 50 mM [^{3}H] dTTP, 0.25 mg/ml of activated calf thymus DNA.

Storage buffer:
50 mM Tris-HCl (pH 8.0 at 25 °C), 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% (v/v) glycerin, and 1% (v/v) Triton X-100.

5× PCR buffer:
50 mM Tris-HCl (pH 8.5 at 25 °C), 250 mM KCl, 0.5% (v/v) Tween 20, stabilizers of *Taq* DNA polymerase.

***Applications*:**
- Hot-start PCR;
- High-throughput PCR;
- Conventional PCR with high reproducibility;
- Synthesis of PCR products for TA cloning;
- RT-PCR.

Limits of use
- Not recommended to use for amplicons of >5 kbp.

Inhibition and inactivation
Inhibitors: ionic detergents (sodium deoxycholate, sarkosyl and sodium dodecyl sulfate (SDS) at concentrations above 0.06, 0.02 and 0.01%, respectively).
Inactivated by phenol/chloroform extraction.

Amplification protocol
Prepare several parallel reactions to minimize possible pipetting error: mix water, buffer, dNTP mix, primers and HS-*Taq* DNA polymerase. Prepare reaction solution by multiplying the total volume by the number of reactions required plus one additional reaction to cover pipetting losses. Aliquot PCR solution to individual PRC tubes, then add DNA template.

1. Thaw the reaction mix and mix carefully.

Note: in case of precipitate formation, heat the tube at 50 °C and stir till complete dissolution.

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

Component	Volume	Final concentration
5× PCR buffer	10 µl	1×
50× dNTP mix	1 µl	0.2 mM of each
50 mM $MgCl_2$*	variable	1-5 mM
Forward primer	variable	0,1 – 300 nM
Reverse primer	variable	0,1 – 300 nM
DNA template	variable	10 pg – 1 µg
HS-*Taq* DNA polymerase, 5 U/µl	variable	1-5 U
Sterile water	up to 50 µl	

3. Carefully vortex and collect all droplets from the tube walls by brief centrifuging.

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. Perform PCR using conditions recommended below:

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5 – 10 sec	25 - 45
Annealing	50 – 68 (Tm-5)	5 - 15 sec	
Elongation	72	1-2 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).

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.