Ver.24021

h-Taq Polymerase

Cat.No. OHT06

DESCRIPTION: h-Taq Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium Thermus aquaticus. Its molecular weight is 94kDa. h-Taq Polymerase can amplify DNA target up to 5kb. The elongation velocity is 2kb/min. It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product. All components of the 10X h-Taq Buffer are at optimal concentration for efficient amplification. It contributes to highly specific incorporation of primer and template.

PRODUCT CONTENTS:

1) h-Taq Polymerase (5U/μL), 250U
2) 10X h-Taq Buffer (Mg^{2+} Plus), 400μL

PROTOCOL

1) Add the following components to a sterile microcentrifuge tube sitting on ice:

a. Recommended PCR assay with 10X h-Taq Buffer (Mg^{2+} Plus)

Reagent	Volume (50μL)	Final Concentration
10X h-Taq Buffer (Mg^{2+} Plus)	5μL	1X
dNTPs (10mM each)	1μL	0.2mM each
Forward Primer	- μL	0.4-1μM
Reverse Primer	- μL	0.4-1μM
h-Taq Polymerase (5U/μL)	0.25-0.5μL	1.25-2.5U/50μL
Template DNA	- μL	10pg-1μg
Add D.W to	50μL	

b. Recommended PCR assay with 10X h-Taq Buffer (Mg^{2+} free)

Reagent	Volume (50μL)	Final Concentration
10X h-Taq Buffer (Mg^{2+} free)	5μL	1X
dNTPs (10mM each)	1μL	0.2mM each
Forward Primer	- μL	0.4-1μM
Reverse Primer	- μL	0.4-1μM
25mM Mg^{2+}	- μL	1-4mM
h-Taq Polymerase (5U/μL)	0.25-0.5μL	1.25-2.5U/50μL
Template DNA	- μL	10pg-1μg
Add D.W to	50μL	

Table for selection volume of 25mM $MgCl_2$ solution in a 50μL reaction mix:

Final Mg^{2+} Con. (mM)	1.0	1.5	2.0	2.5	3.0	4.0
Mg^{2+} (25mM)	2μL	3μL	4μL	5μL	6μL	8μL

Recommendation amounts of template DNA in a 50µL reaction mix:

Human genomic DNA	0.1µg – 1µg
Plasmid DNA	0.5ng – 5ng
Phage DNA	0.1ng – 10ng
E.coli genomic DNA	10ng – 100ng

2) **Mix the contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.**
   When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25µL mineral oil.

3) **Perform 25-35 cycles of PCR amplification as follows:**

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C	30 seconds
	55-68°C	30 seconds
	72°C	1-10 minutes
Final Extension	72°C	10 minutes

4) **Incubate for an additional 10 minutes at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.**

5) **Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.**

APPLICATIONS

- PCR amplification of DNA fragments as long as 5kb
- DNA labeling
- DNA sequencing
- PCR for cloning

STORAGE

Store at -20°C for 12 months.