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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Maxima® Hot Start *Taq* DNA Polymerase

#EP0603 5x500 u

Lot: _ **Expiry Date:** _

Concentration: 5 u/µl

Supplied with: 10x1.25 ml of 10X Hot Start PCR Buffer
10x1.25 ml of 25 mM $MgCl_2$

Store at -20°C

ISO 9001 | ISO 14001
www.fermentas.com

Description

Maxima® Hot Start *Taq* DNA Polymerase is designed to enhance the specificity, sensitivity and yield of DNA amplification (1-4). In addition, the enzyme provides the convenience of reaction set-up at room temperature.

Maxima® Hot Start *Taq* DNA Polymerase is a recombinant *Taq* DNA polymerase which has been chemically modified by the addition of heat-labile blocking groups to its amino acid residues. The functional activity of the enzyme is restored during a short 4-minute incubation at 95°C. The activated enzyme maintains the same functionality as *Taq* DNA polymerase: catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' proofreading exonuclease activity, but possesses low 5'→3' exonuclease activity. It exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Before activation, the two activities are not detectable.

Applications

- Hot start PCR.
- RT-PCR.
- Highly specific amplification of complex genomic and cDNA templates up to 3 kb.
- Amplification of low copy DNA targets.
- Real-time PCR.
- Multiplex PCR.
- Generation of PCR products for TA cloning.

Source

E. coli cells carrying a cloned *pol* gene from *Thermus aquaticus.*

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 74°C.

Enzyme activity is assayed in the following mixture: 25 mM TAPS (pH 9.3 at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dATP, dGTP, dTTP, 0.1mM dCTP, 0.75 mM activated salmon milt DNA and 0.4 MBq/ml [^{3}H]-dCTP. The enzyme is activated by heating for 3 hours at 80°C, before the activity is measured.

Storage Buffer

Enzyme is supplied in: 20 mM Tris-HCl (pH 9.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Tween 20, 0.5% (v/v) Nonidet P40 and 50% glycerol.

10X Hot Start PCR Buffer

200 mM Tris-HCl (pH 8.3 at 25°C), 200 mM KCl, 50 mM $(NH_4)_2SO_4$.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively (5).
- Inactivated by phenol/chloroform extraction.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 1 µg of pUC19 DNA with 25 u of Maxima® Hot Start *Taq* DNA Polymerase in 50 µl of reacture mixture for 17 hours at 37°C and analysis on agarose gel.

Deoxyribonuclease Assay

No detectable oligonucleotide degradation was observed after incubation of single-stranded and double stranded [^{33}P]-labeled oligonucleotides with 10 u of Maxima® Hot Start *Taq* DNA Polymerase for 4 hours at 37°C, analysis on polyacrylamide gel and phosphoroimaging.

Ribonuclease Assay

No decrease in RNA band intensity compared to negative control was observed after incubation of 80 ng of 2 kb RNA transcript with 25 u of Maxima® Hot Start *Taq* DNA Polymerase for 4 hours at 37°C and analysis on agarose gel.

Functional Assay (PCR)

Maxima® Hot Start *Taq* DNA Polymerase was tested in PCR amplification of a 354 bp DNA fragment of single copy ApoE gene from 50 pg of human genomic DNA in a 20 µl PCR mixture containing 1X Hot Start PCR Buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM primers and 0.5 units of Maxima® Hot Start *Taq* DNA Polymerase. The PCR products were analyzed on 1% agarose gel.

Quality authorized by: Jurgita Zilinskiene

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PROTOCOL

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by adding water, buffer, dNTPs, primers and *Taq* DNA Polymerase. Prepare enough master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and add template DNA. Keep all reaction components on ice. Reaction set up can be performed at room temperature.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add the following components for each 50 µl reaction at room temperature:

10X Maxima® Hot Start *Taq* buffer	5 µl
dNTP Mix, 2 mM each (#R0241)	5 µl (0.2 mM of each)
Forward primer	0.1-1 µM
Reverse primer	0.1-1 µM
25 mM $MgCl_2$	1-4 mM*
Template DNA	1 pg - 1 µg
Maxima® Hot Start *Taq* DNA Polymerase	1.25-2 u
Water, nuclease-free (#R0581)	to 50 µl
Total volume	**50 µl**

*Volumes of 25 mM $MgCl_2$ solution to be used:

Final concentration of $MgCl_2$, mM	1	1.5	2	2.5	3	4
Volume of 25 mM $MgCl_2$ for 50 µl reaction, µl	2	3	4	5	6	8

3. Gently vortex and spin down the samples.
4. When using thermal cyclers without a heated lid, overlay the reaction mixture with 25 µl mineral oil.
5. Perform PCR using recommended thermal cycling conditions:

Step	**Temperature, °C**	**Time**	**Number of cycles**
Initial denaturation/ enzyme activation	95	4 min	1
Denaturation	95	0.5-1 min	25-40
Annealing	Tm-5	0.5-1 min	
Extension	72	1 min/kb	
Final extension	72	5-15 min	1

References

1. D'Aquila, R.T., et al., Maximizing sensitivity and specificity of PCR by preamplification heating, Nucleic Acids Res., 19, 3749, 1991.
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3. Horton, R.M., Hoppe, B.L., and Conti-Tronconi, B.M., AmpliGrease: "Hot Start" PCR using petroleum jelly, BioTechniques, 16, 42-43, 1994.
4. Dang, C. and Jayasena S.D., Oligonucleotide inhibitors of *Taq* DNA polymerase facilitate detection of low copy number targets by PCR, J. Mol. Biol., 264, 268-278, 1996.
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