

CERTIFICATE OF ANALYSIS

Maxima[®] Hot Start Taq DNA Polymerase

#EP0601 100 u

Lot: _ **Expiry Date: _**

Concentration: 5 u/μl

Supplied with: 0.6 ml of 10X Hot Start PCR Buffer
0.6 ml of 25 mM MgCl₂

Store at -20°C

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 MgCl₂,

0.2 mM of each dATP, dGTP, dTTP, 0.1 mM dCTP,

0.75 mM activated salmon milt DNA and

0.4 MBq/ml [³H]-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 (NH₄)₂SO₄.

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

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 25 units of Maxima® Hot Start *Taq* DNA Polymerase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Deoxyribonuclease Assay

No degradation of single-stranded and double stranded [³²P]-labeled oligonucleotide was observed after incubation with 10 units of Maxima® Hot Start *Taq* DNA Polymerase for 4 hours at 37°C.

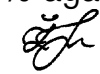
Ribonuclease Assay

No contaminating RNase activity was detected 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.

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 MgCl₂, 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 μ l reaction at room temperature:

10X Maxima[®] Hot Start <i>Taq</i> buffer	5 μ l
dNTP Mix, 2 mM each (#R0241)	5 μ l (0.2 mM of each)
Forward primer	0.1-1 μ M
Reverse primer	0.1-1 μ M
25 mM MgCl₂	1-4 mM*
Template DNA	1 pg - 1 μ g
Maxima[®] Hot Start <i>Taq</i> DNA Polymerase	1.25-2 u
Water, nuclease-free (#R0581)	to 50 μ l
Total volume	50 μl

*Volumes of 25 mM MgCl₂ 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 μ l reaction, μ l	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 μ l 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	T _m -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.
2. Kellog, D.E., et al., TaqStart antibody: "Hot start" PCR facilitated by a neutralizing monoclonal antibody directed against *Taq* DNA polymerase, *BioTechniques*, 16, 1134-1137, 1994.
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.
5. Weyant, R.S., et al., Effect of ionic and nonionic detergents on the *Taq* polymerase, *Biotechniques*, 9, 309-308, 1990.

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