CERTIFICATE OF ANALYSIS

Maxima™ Hot Start *Taq* DNA Polymerase

#EP0601 100u

Lot: **Expiry Date:**

Concentration: 5u/µl

Supplied with: 0.6ml of 10X Hot Start PCR Buffer
0.6ml of 25mM $MgCl_2$

Store at -20°C

In total 3 vials.

ISO 9001 ISO 14001
www.fermentas.com

Description

The Maxima™ Hot Start *Taq* DNA Polymerase is designed for Hot Start PCR, a technique that enhances the specificity, sensitivity and yield of DNA amplification (1-4). In addition, the enzyme provides the convenience of reaction set-up at room temperature.
The 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 amino acid residues. The enzyme is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers. The functional activity of the enzyme is restored during a short 4-minute incubation at 95°C. The activated enzyme is a functional equivalent of *Taq* DNA Polymerase: it catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' proofreading exonuclease activity, but possesses low 5'→3' exonuclease activity.

Applications

Hot Start PCR with genomic and cDNA targets, RT-PCR, amplification of low copy DNA targets, real-time PCR.

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 10nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 70°C.

Activity Assay

67mM Tris-HCl (pH 8.8 at 25°C), 6.7mM $MgCl_2$, 1mM 2-mercaptoethanol, 50mM NaCl, 0.1mg/ml BSA, 0.75mM activated calf thymus DNA, 0.2mM of each dNTP, 0.4MBq/ml [^{3}H]-dTTP. The enzyme is activated by heating for 3 hours at 80°C before the activity is measured.

Storage Buffer

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

10X Hot Start PCR Buffer

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

Inhibition and Inactivation

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 10 units of Maxima™ Hot Start *Taq* DNA Polymerase with 1µg of pUC19 DNA in 50µl of Hot Start PCR buffer containing 2.0mM $MgCl_2$ for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0.06% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of Maxima™ Hot Start *Taq* DNA Polymerase with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of Hot Start PCR buffer containing 2.0mM $MgCl_2$ for 4 hours at 37°C.
0.06% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of Maxima™ Hot Start *Taq* DNA Polymerase with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of Hot Start PCR buffer containing 2.0mM $MgCl_2$ for 4 hours at 65°C.

Ribonuclease Assay

0.1% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 units of Maxima™ Hot Start *Taq* DNA Polymerase with 1µg of [^{3}H]-RNA in 50µl of Hot Start PCR buffer containing 2.0mM $MgCl_2$ for 4 hours at 37°C.

Functional Assay

Maxima™ Hot Start *Taq* DNA Polymerase was tested for yield and specificity in amplification of a 950 bp DNA fragment of the single copy gene using human genomic DNA as template.

Quality authorized by: Jurgita Zilinskiene

(For STANDARD PROTOCOL *see* back page)

STANDARD PROTOCOL

The standard protocol is proposed as a guideline. Individual reactions require optimization of $MgCl_2$, primer, template and Maxima™ Hot Start *Taq* DNA polymerase concentrations as well as optimization of cycling temperatures and times. Refer to www.fermentas.com for detailed protocol.

Reaction Set-up

- Gently vortex and briefly centrifuge all solutions after thawing to collect the contents.
- Add in a thin-wall PCR tube at room temperature:

Component	Volume	Final concentration
Water, nuclease free	variable	
10X Hot Start PCR buffer	5µl	1X
dNTP mix, 2mM each	5µl	0.2mM each
Primer I	variable	0.1-1µM
Primer II	variable	0.1-1µM
25mM $MgCl_2$	variable*	1.5-4mM
Hot Start *Taq* DNA Polymerase	0.25-0.4µl**	1.25-2.0u/50µl
Template DNA	variable	10pg-0.5µg/50µl
Total volume:	**50µl**	

*To reach final concentration of $MgCl_2$ in the 50µl reaction volume, add aliquots of 25mM $MgCl_2$ solution (supplied):

Final concentration	1.5mM	1.75mM	2.0mM	2.5mM	3.0mM	4.0mM
Volume of 25mM $MgCl_2$	3µl	3.5µl	4µl	5µl	6µl	8µl

**1.25 units is sufficient for amplifying most targets. Increasing Maxima™ Hot Start *Taq* DNA Polymerase concentration may result in higher yields of specific product.

Note

To achieve 0.2mM concentration of each dNTP in the PCR mixture, use the following volumes of dNTP Mixtures from Fermentas:

Volume of PCR mixture	dNTP Mix, 2mM each #R0241	dNTP Mix, 10mM each #R0191	dNTP Mix, 25mM each #R1121
50µl	5µl	1µl	0.4µl
25µl	2.5µl	0.5µl	0.2µl

- Gently vortex and briefly centrifuge to collect drops. For thermal cyclers without a heated lid, overlay the reaction mixture with 25µl of mineral oil.

Reaction volumes can be scaled up or down as long as final concentrations of reaction components remain the same.

Thermal Cycling Conditions

Step	Temperature	Time	Number of cycles
Initial denaturation/ enzyme activation	95°C	2-4min	1
Denaturation	95°C	0.5-1min	25-40
Annealing	37-68°C	0.5-1min	
Extension	68-72°C	1min/kb	
Final extension	68-72°C	5-15min	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.

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Related Products

- dNTP Mix, 2mM each #R0241, #R0242
- dNTP Mix, 10mM each #R0191, #R0192
- dNTP Mix, 25mM each #R1121, #R1122
- dNTP Set #R0181
- Water, nuclease-free #R0581, #R0582
- FastRuler™ Low Range DNA Ladder #SM1103
- FastRuler™ Middle Range DNA Ladder #SM1113
- GeneRuler™ Express DNA Ladder #SM1551, #SM1552, #SM1553
- GeneRuler™ 100bp DNA Ladder #SM0241, #SM0242, #SM0243
- GeneRuler™ 100bp Plus DNA Ladder #SM0321, #SM0322, #SM0323
- ΦX174 DNA/BsuRI Marker, 9 #SM0251, #SM0252, #SM0253
- 25mM $MgCl_2$ #R0971
- CloneJET™ PCR Cloning Kit #K1231, #K1232
- Genomic DNA Purification Kit #K0512
- DNA Gel Extraction Kit #K0513

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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