PRODUCT INSERT

PUREGENE GENETIX BRAND

GeneMax Hot Start Taq DNA Polymerase

Store at -20°C

Cat. No.
PGP0206

Pack Size
500 Units

Concentration: 5 U/µL
Supplied with: 2x1.25 mL of 10X Hot Start PCR Buffer
(Includes 20 mM $MgCl_2$)

Storage: -20°C

Description
Puregene GeneMax 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. GeneMax 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 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 in 30 min at 74°C.

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.

CERTIFICATE OF ANALYSIS
Endodeoxyribonuclease Assay
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 25 units of GeneMax 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 [33P]-labeled oligonucleotide was observed after incubation with 10 units of GeneMax 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 GeneMax Hot Start Taq DNA Polymerase for 4 hours at 37°C.

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Functional Assay (PCR)

GeneMax 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 GeneMax Hot Start Taq DNA Polymerase. The PCR products were analyzed on 1% agarose gel.

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 Hot Start *Taq* buffer	5 µL
dNTP Mix, 2 mM each	5 µL (0.2 mM of each)
Forward primer	0.1-1 µM
Reverse primer	0.1-1 µM
Template DNA	10 pg - 1 µg
GeneMax Hot Start Taq DNA Polymerase	1.25-2 U
Water, nuclease-free	to 50 µL
Total volume	**50 µL**

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