

HotStart Tag DNA Polymerase

Cat: PE3009 Size: 250 Units Conc. 5 units/µl Store at -20°C.

Description

HotStart Taq DNA Polymerase is a high purity, recombinant Taq DNA polymerase preparation with high avidity monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94°C), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly. Non-specific extension of primers at low temperatures is a common cause of artifacts and poor sensitivity in PCR. The HotStart Polymerase automatic hot-start enables specific and efficient primer extension in the PCR process with the added convenience of room temperature reaction assembly. The included 10X PCR Buffer II is a new optimized buffer that provides higher product yield, improved specificity, and enhanced multiplexing capability. Activated HotStart Taq DNA Polymerase possesses 5′->3′DNA polymerase activity and a double-strand specific 5′->3′exonuclease. The polymerase does not have 3′-exonuclease activity and is free of any contaminating endo or exonuclease activities. One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acidinsoluble material in 30 minutes at 74°C. HotStart Taq DNA Polymerase contains extremely low levels of residual host, *E. coli* genomic DNA.

Contents:

HotStart Taq DNA Polymerase 5 units/ μ L in 50% glycerol, 20 mM Tris-HCl, 40 mM NaCl, 0.1 mM EDTA, and stabilizers.

10X PCR Buffer II Optimized 10X-concentrated buffer 50 mM MgCl₂

Storage conditions

HotStart Taq DNA Polymerase is stable for 3 years when stored in a constant temperature freezer at -20°C.

Preparation of PCR reaction mixture:

Components	Qty.	Final concentration	
Template DNA	variable	10-250 ng for genomic DNA template	
		0.1-10 ng for plasmid DNA template	
Forward primer, 10 □M	0.5-2.5 ul	0.1-0.5 uM	
Reverse primer, 10 □M	0.5-2.5 ul	0.1-0.5 uM	
dNTP mix (10 mM each)	1 ul	200 uM each	
PCR Buffer, 10X	5 ul	1X	
MgCl ₂	1-5 ul	1-5 mM	
Taq DNA Polymerase	0.2 ul	1.0 unit	
ddH₂O	up to 50 ul	Variable	
Final volume	50 ul		



Program of Thermal cycling:

Step 1	94°	1-3 min	92°, 95°, 94° are the standard denaturation
Initial denaturation			temperatures
Step 2	94°	15-30	
Denaturation		sec	
Step 3	55°- 65°	30 sec	Optimal annealing temperature depends on
Annealing			primers Tm and the reaction conditions.
Step 4	68-72°	1	Adjust elongation time based on product size
Elongation		min/kb	(1kb per 1 min)
Step 5	Go to Step 2		
Recycle	20-40 cycles		
Step 7	4-15°	∞	
Hold			

Accessory Products

The following products are available separately from Genbiotech.

Product	Quantity	Catalog no.
dNTPs in Separate Tube (100 mM each)	4 x 100 ul	PD2001
dNTPs in Separate Tube (100 mM each)	4 x 250 ul	PD2002
dNTPs in Separate Tube (100 mM each)	4 x 400 ul	PD2003
EvaGreen [™] , 20X in water	5 x 1 ml	31001
Custom oligonucleotides		