Hot Start Taq DNA Polymerase
Cat. No. E00049 Version 06152017

I Description

Hot Start Taq DNA Polymerase is a recombinant, thermostabile Taq DNA polymerase complexed with a thermolabile, neutralizing antibody that blocks the polymerase activity prior to the initial DNA denaturation step of PCR. Such antibody-mediated Hot-Start capability enhances the overall specificity, sensitivity and yield of the PCR by reducing nonspecific amplification and primer-dimer formation prior to PCR cycling, and allows the convenience of reactions set up at room temperature. When the temperature of the PCR reaction mix reaches 95°C during the initial DNA denaturing step of PCR cycling, activity of the Taq DNA polymerase is fully restored.

II Kit Contents

Kit Contents	Quantity	Catalog No.	Components/Concentration
Hot Start Taq DNA Polymerase	250 U	E00049-250	5 U/μL
	1000 U	E00049-1000	5 U/μL
10×Taq Buffer	1mL		10×

III Storage

Please store at -20°C.

IV Product Usage

Hot Start Taq DNA Polymerase is used for PCR amplification with enhanced specificity.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

V Protocol

Ensure all components are thawed and mixed well. If preparing multiple reactions, assemble all common components into a master mix. If working with final reaction volumes less than 50 µL, scale component volumes accordingly.

10×Taq Buffer	5 µL
dNTP Mix (10 mM each)	1 µL
5' primer, 10 µM	1 µL
3' primer, 10 µM	1 µL
Hot Start Taq DNA Polymerase§	0.2-0.5 µL (1-2.5 U)
DNA template（10-100 ng）	X µL
Nuclease free H_2O	Up to 50 µL

§ Typically 1 U, may need up to 2.5 U for fragments > 8kb

PCR cycling parameters:

Steps	Temperature	Time	Cycles
Initial Denaturation	95 ℃	30 s or 5 min	1
Denaturation	94 ℃	15-30 s	25-35
Annealing	Varies	15-30 s	
Extension	72 ℃	1 kb/min	
Final Extension	72 ℃	5 min	1
	4 ℃	Hold	-

Note： This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.

VI Quality Control Analysis

- **Antibody Inhibition Assay:** Greater than 95% inhibition is observed after a 30 minute incubation at 65°C.
- **Antibody Heat Inactivation Assay:** Greater than 95% activity of Taq DNA polymerase is reversed after a 30 second incubation at 95°C.
- **RNase activity assay:** Incubation of a 10 µL reaction containing 10 units of Hot Start Taq DNA Polymerase with 1 µg total RNA for 1 hour at 37°C doesn't result in the degradation of total RNA as determined by agarose gel electrophoresis.
- **DNase activity assay:** Incubation of a 25 µL reaction containing 10 units of Hot Start Taq DNA Polymerase with 500 ng DNA for 16 hours at 37°C doesn't result in the degradation of DNA as determined by agarose gel electrophoresis.

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VII Activity Test

1. To test the blocking activity of Hot Start Taq antibody, a primer extension assay was done as follows. A pair of primers was designed with a 14 bp overlap. One is 24 bp and the other one is 41 bp. The primers were annealed and incubated with Taq DNA polymerase (lane 1-2) or Hot Start Taq DNA polymerase (lane 3-4) for 0.5 h at 65℃. The extension result was separated on an Urea PAGE gel. No primer dimers were observed with Hot Start Taq polymerase.

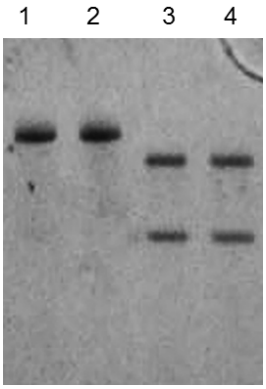

Figure 1. The primer extension assay to detect the blocking activity of Taq Antibody.

2. To test the specificity of amplification, a 500 bp long HPRT fragment was amplified from human genomic DNA using Hot Start Taq DNA polymerase (lanes 1-2) and Taq DNA polymerase (lane 3-4). No non-specific bands were observed in the amplification reaction with the Hot Start Taq DNA polymerase.

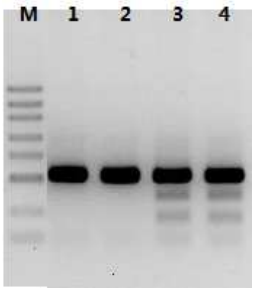

Figure 2. The PCR specificity detection of Hot Start Taq DNA polymerase.

GenScript US
860 Centennial Ave., Piscataway, NJ 08854
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Email: product@genscript.com
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