Applied Biological Materials Inc.

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Hotstart Taq DNA Polymerase

Store at -20°C

Cat. No.	Conc.	Quantity
G011	5U/µl	250U
G039	5U/µl	1,000U

Description

Hotstart Taq DNA Polymerase is a chemically modified Taq DNA Polymerase whose enzyme activities can only be activated after 3-5 minutes of incubation at 94°C. This enzyme thus exhibits no polymerase activities before the onset of thermal cycling, preventing nonspecific DNA amplification and primer dimer formation. PCR products, amplified up to 6kb in length with Taq DNA Polymerase, generate a single base (A) 3′ overhang.

Source

E.coli cells carrying a cloned *pol* gene from *Thermus aquaticus*.

Components	250U	1,000U
Hotstart Taq DNA Polymerase	50µl	200µl
10X PCR buffer, minus Mg	1.0ml	2.0ml
25mM $MgCl_2$	1.0ml	1.0ml

Storage Buffer*

50mM Tris-HCl (pH 8.0), 100mM NaCl, 0.1mM EDTA, 5mM DTT, 50% glycerol and 1.0% Triton7X-100.

10X PCR Buffer*

200mM Tris-HCl (pH 8.4), 200mM KCl, 25mM $MgCl_2$ supplied separately.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction in 30 mins at 70°C.

*Intellectual property included

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of Taq Polymerase, primers, $MgCl_2$ and template DNA) vary and need to be optimized.

PCR reactions should be assembled in a DNA-free environment. DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction analysis, should be performed in separate areas.

A control reaction, omitting template DNA, should always be performed to confirm the absence of contamination.

1. Add the following components to a sterile 0.2ml PCR tube sitting on ice.

Components	Volume
Template DNA	<1µg
Forward Primer 1 (10µM)	1-3µl
Reverse Primer 2 (10µM)	1-3µl
10X PCR Buffer	5µl
25mM $MgCl_2$	3µl
dNTP Mixture (10 mM each)	1µl
Hotstart Taq Polymerase (5U/µl)	0.5-1µl
ddH_2O	up to 50µl

We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.

2. Mix contents of tube and centrifuge briefly.
3. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.
4. Perform 25-35 cycles of PCR amplification as follows:

   Denature: 94°C for 30 sec
   Anneal: 55°C for 30 sec
   Extend: 72°C for 1 min/1kb template

5. Incubate for an additional 10 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ staining. Use appropriate molecular weight standards.

This product is distributed for laboratory research only.
CAUTION: Not for clinical use.
For technical questions about this product,phone the ABM helpline at 1-866-571-7226 or visit our website at www.abmGood.com.

CERTIFICATE OF ANALYSIS