GENTAUR

DATA SHEET

HotStart Taq DNA Polymerase

Store at -20°C

Cat. No.	Description	Concentration	Quantity
G011	HotStart Taq DNA Polymerase	5 U/µl	250 U
G039	HotStart Taq DNA Polymerase	5 U/µl	1000 U

Product 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 non-specific DNA amplification and primer dimer formation. PCR products, amplified up to 6 kb in length with HotStart Taq DNA Polymerase, contain a single base (A) 3' overhang.

Product Components	250 U	1000 U
HotStart Taq DNA Polymerase (5 U/µl)	50 µl	200 µl
10X PCR buffer, with Mg^{2+}	1 ml	3 ml
25 mM $MgSO_4$	1 ml	1 ml

Storage Buffer Components
50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50 % glycerol and 1.0 % Triton®X-100.

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

Shipping and Storage
Upon arrival, HotStart Taq DNA Polymerase should be stored at -20°C. Avoid repeated freeze-thaw cycles of all HotStart Taq components to retain maximum performance. All HotStart Taq components are stable for 1 year from the date of shipping if stored and handled properly.

Protocol
The following basic protocol serves as a general guideline and starting point for any PCR amplification. Optimal reaction conditions (incubation times, temperatures, concentration of Taq DNA Polymerase, primers, $MgSO_4$ and template DNA) may vary and need to be optimized for each specific PCR.

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross contamination.

A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

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

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µl
Forward primer (10 µM)	1 - 2.5 µl	200 - 500 nM
Reverse primer (10 µM)	1 - 2.5 µl	200 - 500 nM
10X PCR buffer, with Mg^{2+}	5 µl	1X
25 mM $MgSO_4$ (optional)*	0 - 3 µl	1.5 - 3 mM
dNTP Mix (10 mM)	1 µl	200 µM
HotStart Taq DNA Polymerase (5 U/µl)	0.5 - 1 µl	2.5 - 5 U
Nuclease-free H_2O	up to 50 µl	-

* Optimal Mg^{2+} concentration is specific to each DNA template-primer set and can only be determined experimentally.

- *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 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 30 sec

   Anneal: 45 - 72°C for 30 sec

   Extend: 72°C for 1 min/1 kb template

5. Incubate for an additional 5 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™ (Cat No. G108) staining. Use appropriate molecular weight standards.

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