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HotStart *Taq* DNA Polymerase

Catalog Number: D007-1, D007-2

Table 1. Kit Components and Storage

Kit Component	D007-1 (200 units)	D007-2 (500 units)	Storage	Stability
HotStart *Taq* DNA Polymerase (5 U/µL)	40 µL	100 µL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.
10× Reaction Buffer	1.5 mL	1.5 mL		

Product Description

The 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. The HotStart *Taq* Polymerase uses amplification conditions for regular *Taq* DNA Polymerase, except no polymerase activity will be present before the onset of thermal cycling. This prevents nonspecific DNA amplification and primer dimer formation. The amplified products, up to 6 kb in length, contain single 3'-overhanging-A ends. This allows for TA cloning methods, if the amplified fragments need to be cloned.

The enzyme is available in 200 and 500 unit sizes at a concentration of 5 U/µL. The enzyme is supplied with a 10× Reaction Buffer.

Special Features

- Low non-specific amplification
- Generate fragments with high specificity
- Sensitivity to low-concentration templates
- Ideal for difficult templates

Applications

- Amplification of genomic DNA and cDNA targets
- Amplification of problematic templates
- General PCR applications

Product Specifications

- **Storage Buffer:** 20 mM Tris-HCl (pH 8.0 at 25°C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.5% (v/v) NP-40, and 50% (v/v) glycerol.
- **Unit Definition:** One unit is defined as the amount of enzyme required to incorporate 10 nmoles of deoxyribonucleotide into DNA in 30 min at 74°C.
- **Unit Assay Conditions:** 25 mM TAPS (pH 9.3), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 mg/ml activated salmon sperm DNA, 0.2 mM dATP, dCTP, dGTP, dTTP.
- **10× Reaction Buffer:** 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 25 mM $MgCl_2$, and other components.

General 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* DNA polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized. Critical parameters and troubleshooting information are documented in reference 1.

Assemble PCR reactions in a nuclease-free environment. Use of “clean” dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes. (End user supplies Primers, and dNTP.)
2. Prepare the following reaction mixture in a PCR tube on ice:

Component	Volume	Final Concentration
Template DNA	x µL	1-500 ng
10× Reaction Buffer	5 µL	1×
10 mM dNTP Mix	1 µL	0.2 mM each
HotStart Taq DNA Polymerase (5 U/µL)	0.2 µL	1-2.5 U/rxn
Forward Primer (10 µM)	2.5 µL	500 nM
Reverse Primer (10 µM)	2.5 µL	500 nM
Nuclease-free H_2O	to 50 µL	-

3. Mix, and then briefly centrifuge the contents.
4. Program the thermal cycler for PCR amplification.

Steps	Temperature	Duration	Cycle
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	45 sec	25-35
Annealing*	55°C (depending on the primer T_m)	30 sec	
Extension	72°C	1 kb/min	
Final Extension	72°C	10 min	1
Holding	4°C	-	1

Note: The thermal cycling program listed above is optimized for primers with an annealing temperature at 55°C.

5. Analyze the amplification products by agarose gel electrophoresis.

Reference

Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, eds. (1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA.