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Product Information

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Product: **HotStart Taq DNA Polymerase**
Code: A4144
Concentration: 5 u/µl

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 ºC. This enzyme, thus, inhibits polymerase reaction before the onset of thermal cycling, reducing nonspecific DNA amplification and primer dimmer formation. The amplified products, up to 6kb in length, contain single 3'-overhanging-A ends. This allows for TA cloning methods, if the amplified fragments need to be cloned. HotStart Taq DNA Polymerase provides a convenient solution for problematic template or primer PCR systems.

Quality Control: HotStart Taq DNA Polymerase is highly purified and free of contaminating endonucleases, exonucleases and nicking activity. For endonuclease assay, 1ug of Lamda/Hind III DNA is incubated with 20 units of enzyme in assay buffer at 75 °C for 16 hours with no visible contaminating activity observed. Also, every lost is tested for its performance consistency.

Unit Definition: One unit incorporates 10 nmol of total dNTPs into acid-insoluble material in 30 min at 70 °C with activated salmon sperm DNA as template.

Storage Buffer: 50mM Tris-HCl (pH 8.0, 22 °C), 100mM NaCl, 0.1mM EDTA, 5mM DTT, 50% glycerol, 1.0% Triton® X-100.

10 x PCR Buffer: 200mM Tris HCl (pH 8.4), 200mM KCl. The Buffer is optimized for use with 200µM dNTPs.

Magnesium Sulfate: 25mM $MgSO_4$. In general 2mM Mg $MgSO_4$ is recommended to start; this may vary with different conditions and primer sets.

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
DNA amplification without non-specific DNA amplification and primer dimmer formation
General PCR, Hot start PCR, Low copy PCR and Multiplex PCR
Generation of PCR products for TA Cloning

Storage: -20 °C

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 EasyStain II (Cat No. A4205) staining. Use appropriate molecular weight standards