

Hot Start-Polymerase

DESCRIPTION

Hot Start DNA Polymerase is recommended for high specificity PCR reactions. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR set up.

Hot Start DNA Polymerase is a highly purified recombinant thermostable DNA polymerase that has been isolated from *E. coli* carrying a vector encoding the *Thermus aquaticus* DNA polymerase gene. The enzyme possesses a highly processive 5'-3' DNA polymerase activity with optimum activity achieved at 74°C. It exhibits high thermal stability in withstanding prolonged incubations at elevated temperatures (95°C). Hot Start DNA Polymerase lacks 3'-5' exonuclease activity.

Red Hot Start DNA Polymerase contains an inert red dye which allows on line monitoring of the electrophoresis and identification of the reactions which contain enzyme. The dye has no adverse effect on PCR.

Activation step

Hot Start Polymerase requires no prolonged heating or denaturing step. The antibodies are quickly inactivated at the increased temperature of thermal cycling.

Unit definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 74°C in the presence of the reaction buffer.

10 x Reaction Buffer

100 mM Tris-HCl pH 8.3 (at 25 °C), 500 mM KCl, 0.1% Triton® X-100

Mg²⁺Solution

50 mM MgCl₂ (recommended final concentration: 1 – 4 mM).

Storage

Store Hot Start DNA Polymerase at -20 °C in a constant temperature freezer. If stored as recommended it will remain stable at least for 12 months.

Endonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 15 units of Hot Start DNA Polymerase with 0.5 µg of pUC 19 plasmid DNA in 10 µl of 1x Reaction Buffer containing 1.5 mM MgCl₂ for 2 hours at 72°C.

Exonuclease Assay

No detectable degradation (smearing) of fragments was observed after incubation of 5 units of Hot Start DNA Polymerase with 0.5 µg of pUC 19 plasmid DNA (digested with Hpa II) in 10 µl of 1x Reaction Buffer containing 1.5 mM MgCl₂ for 2 hours at 72°C.

Ribonuclease Assay

No detectable degradation of 28S/18S bands was observed after incubation of 5 units of Hot Start DNA Polymerase with 1 µg of total RNA (from rat liver) in 10 µl of 1x Reaction Buffer containing 1.5 mM MgCl₂ for 2 hours at 72°C.

Functional Assay

Hot Start DNA Polymerase was serially diluted and tested for amplification of a 4 kbp fragment of Lambda DNA.

BASIC PROTOCOL

Mix the following components on ice in a thin-walled 0.2 ml PCR-tube:

Component	Volume	Final concentration
Double distilled, sterile water	variable (added to a final volume of 50 µl)	
10x Reaction Buffer	5 µl	1 x
50 mM MgCl ₂ Solution	1.5 µl	1.5 mM
12.5 mM dNTP-Mastermix	1 µl	250 µM
Forward Primer	variable	0.2 – 1 µM
Reverse Primer	variable	0.2 – 1 µM
Template DNA	variable	<1 µg
Hot Start DNA Polymerase [5 U/µl]	0.2 – 0.5 µl	1 – 2.5 U

HINTS AND NOTES

- Mix the $MgCl_2$ solution before use by vortexing vigorously.
- Spin down all components before use.
- Mix the components of the reaction after pipetting by vortexing shortly. Then collect the mixture at the bottom of the tubes by centrifugation for some seconds.
- Keep the reaction tubes on ice as long as possible. Transfer the tubes from the ice to the cyclor immediately after the denaturation temperature of about $94^\circ C$ has been reached.

Reaction conditions (incubation temperatures and times, concentrations of template DNA, primers, magnesium ions and enzyme) depend on template and primers used. Optimal $MgCl_2$ concentrations vary between 1-4 mM and have to be determined empirically. However, many applications work at the standard concentration of 1.5 mM $MgCl_2$. Advanced applications on genomic DNA require higher $MgCl_2$ concentrations (2-3 mM) adjustable with the separate 50 mM $MgCl_2$ solution supplied with the set.

OPTIMIZATION OF $MgCl_2$ CONCENTRATION IN REACTION MIXTURE

Final concentration of $MgCl_2$ in 50 μl reaction volume	1.5 mM	1.5 mM	2 mM	2.5 mM	3 mM	4 mM
Add 50 mM $MgCl_2$ solution	1.5 μl	1.75 μl	2 μl	2.5 μl	3 μl	4 μl

WARNING

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.