Hotstart Pfu Mix

Cat. No.: P2051, P2052

Contents:

Component	P2051	P2052
2X Hotstart Pfu Mix	1 ml	1 ml × 5
Nuclease-free water	1 ml	1 ml × 5

Store at -20°C

Description

2X Hotstart Pfu Mix is a premixed, ready-to-use solution containing Hotstart Pfu DNA Polymerase, dNTPs, Mg^{2+} and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only need to add primers and template DNA. This premixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set-up. The mix retains all features of Hotstart Taq DNA Polymerase. Hotstart Pfu DNA Polymerase is a hot-start polymerase with antibody modification, which brings higher specificity by reducing non-specific products as the enzyme activity is temperature-dependent and is inhibited at room temperature. The amplification length can reach to 5 kb (simple template) with the elongation rate of 2kb/min. Hotstart Taq has 5'-3' polymerase activity and 3'-5' exonuclease activity. There are blunt-end and 3'dA-end two kinds of products. Hotstart Pfu DNA Polymerase has zero animal source pollution by being produced with advanced antibody modification. And it is much more stable than antibody-modified hot-start polymerase. Its efficiency is higher than most chemical-modified polymerases and the initial-denaturation time can be reduced to 3 minutes.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Composition of the 2X Hotstart Pfu Mix

0.5U/ul Hotstart Pfu DNA Polymerase, 2× PCR buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$, 0.02% bromophenol blue (optional).

Applications

- High-specificity amplification & multiplex PCR: hot-start polymerase with antibody modification
- High fidelity PCR

Basic PCR Protocol

1. Add the following components to a sterile microcentrifuge tube sitting on ice (not necessary):

Reagent	Quantity	Final concentration
2X Hotstart Pfu Mix	25 µl	1X
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10pg-1µg
Water, nuclease-free	to 50 µl	–

Recommendation amounts of template DNA in a 50 µl reaction mix:

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

2. Mix contents in the tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	95°C	2 minutes
25-35 Cycles	95°C	10 seconds
	55-68°C	20 seconds
	72°C	1-2 minutes
Final Extension	72°C	5-10 minutes

4. Maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- Hotstart Pfu DNA Polymerase adopts improved antibody modification technology, so it relies on temperature to activate the polymerase activity, which can effectively inhibit non-specific bindings.
- To avoid primer degradation due to enzyme correction activity, prepare the reaction system on ice.
- Hotstart Pfu DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.

- dUTP, dITP and primers containing these nucleotides cannot be used for PCR amplification catalyzed by Pfu DNA polymerase. Because when the enzyme binds to a DNA template containing uracil or hypoxanthine, it stops the DNA polymerization process.

Guidelines for preventing contamination of PCR

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set-up.
- Always perform “no template control” (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25 µl Hotstart Pfu Mix (2X) with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25 µl Hotstart Pfu Mix (2X) with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µl Hotstart Pfu Mix (2X) with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.