

High Fidelity DNA-Polymerase

DESCRIPTION

Pfu DNA Polymerase is a highly purified recombinant highly thermostable DNA polymerase that has been isolated from *E. coli* carrying a vector encoding the *Pyrococcus furiosus* DNA polymerase gene. The enzyme is twenty-five times more accurate than Taq DNA Polymerase and it produces blunt-end amplicons.

Storage buffer

20mM Tris/HCl (pH 8.2 at 25°C), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.1% (v/v) Tween 20, 50% (v/v) glycerol.

10x Reaction Buffer

500mM Tris/HCl, 140mM (NH₄)₂SO₄, 17.5mM MgCl₂, (pH 9.1 at 25°C).

Unit Definition

One unit catalyzes the incorporation of 10nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30min at 72°C.

Endonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 15 units of pfu/High Fidelity 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 pfu/High Fidelity 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 pfu/High Fidelity 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

pfu DNA Polymerase and High Fidelity DNA Polymerase were tested for amplification of a 20 kbp fragment of human genomic DNA.

BASIC PROTOCOL

Component	Volume in μ l
10x Buffer	5
10 mM dNTP Mix	1
Primer 1 (10 pmol/ μ l)	2
Primer 2 (10 pmol/ μ l)	2
Template	x
5x Band Doctor	0 - 20
Pfu / HF Polymerase (2.5U/ μ l)	0.5
D.W.	to 50

Cycling Conditions

Temperature	Time	Cycles
95° C	2 min	1
95° C	20 sec	20 - 40
AT*	40 sec	20 - 40
72° C	see chapter E	20 - 40
72° C	5 min	1

AT* : Annealing Temperature; Adjust to the lower T_m between two primers; $AT = T_m - (4-6^\circ C)$;
 $T_m = 2^\circ C \times (A+T) + 4^\circ C \times (G+C)$

High Fidelity DNA Polymerase is a blend of DNA polymerases specially designed for the highly accurate and efficient amplification of fragments up to 30kbp including GC-rich or other difficult templates.

APPLICATION

A Template (temperature cycle)

Animal genomic DNA:

50-200 ng (25-35 cycles)

0-50 ng (30-40 cycles)

Bacterial genomic DNA:

10-50 ng (20-25 cycles)

1-5 ng (30-35 cycles)

Plasmid and lambda DNA:

1-5 ng (20-30 cycles)

B. DNA Polymerase

For amplification of longer fragments of an animal genomic DNA, the amount of enzyme should be increased to 2 or 2.5 U.

C. 5x Band doctor

The use of Band doctor is not necessary in general, but helps in the amplification of DNA with high GC content and complex structures. The included 5x Band doctor can be added to the reaction mixture at the final concentration of 0.5x – 2x.

Reaction mixture (conc. of band doctor)

Component	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
10x Pfu Buffer	5 µl	5 µl	5 µl	5 µl	5 µl
10 mM dNTP Mix	1 µl	1 µl	1 µl	1 µl	1 µl
Primer 1 (10 pmol/µl)	2 µl	2 µl	2 µl	2 µl	2 µl
Primer 2 (10 pmol/µl)	2 µl	2 µl	2 µl	2 µl	2 µl
Template	x µl	x µl	x µl	x µl	x µl
5x Band Doctor	0 µl	5 µl	10 µl	15 µl	20 µl
Pfu/HF (2.5U/µl)	0.5 µl	0.5 µl	0.5 µl	0.5 µl	0.5 µl
add to D.W. to	50 µl	50 µl	50 µl	50 µl	50 µl

D. Primer design

Primers can be designed manually or using a “primer design software”. It is recommended that T_m of the designed primers is above 64°C, and AT, above 58°C.

E. Extension time

The extension time should be 2 min/kb for Pfu Polymerase.

For High Fidelity Polymerase the extension time should be 30 sec/kb for short fragments and 1 min/kb for fragments longer than 5 kb.

For the amplification of fragments longer than 5 kb, the temperature ought to be assigned at 68°C.

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.