Pfu DNA Polymerase

#P1021, 250U

Concentration: 2.5U/μl
Contents:

Pfu DNA Polymerase	100 μl
10× Pfu Buffer (Mg^{2+} Plus)	1.25 ml
6× Loading Buffer	1 ml

Store at -20°C

For research use only.

In total 3 vials.

Description

Pfu DNA polymerase, derived from the hyperthermophilic archae Pyrococcus furiosus, has superior thermostability and proofreading properties compared to other thermostable polymerase. Its molecular weight is 90 kDa. It can amplify DNA target up to 2 kb （simple template）. The elongation velocity is 1kb/min(70~75°C). Pfu DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that Pfu DNA polymerase-generated PCR fragments will have fewer errors than Taq-generated PCR inserts. Using Pfu DNA polymerase in your PCR reactions results in blunt-ended PCR products, which are ideal for cloning into blunt-ended vectors. Pfu DNA polymerase is superior for techniques that require high-fidelity DNA synthesis.

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.

Storage Buffer

20mM Tris-HCl (pH 8.0), 100mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (V/V) glycerol.

10× Pfu Buffer with Mg2+

200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM $(NH_4)_2SO_4$, 20mM

$MgSO_4$, 1% Triton X-100, 1mg/ml BSA.

Applications

- High-fidelity PCR and primer-extension reactions
- High fidelity PCR for cloning into blunt-ended vectors
- Site-directed mutagenesis

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

1.1 Recommended PCR assay with Pfu Buffer (Mg^{2+} plus)

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× Pfu Buffer (Mg^{2+} plus)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Pfu DNA Polymerase (2.5U/µl)	0.5-1.0 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

1.2 Recommended PCR assay with Pfu Buffer (Mg^{2+} free)

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× Pfu Buffer (Mg^{2+} free)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
25mM Mg^{2+}	variable	1.0-4.0mM
Pfu DNA Polymerase (2.5U/µl)	0.5-1.0 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

Table for selection volume of 25 mM $MgCl_2$ solution in a 50 µl reaction mix:

Final Mg^{2+} Conc. (mM)	1.0	1.5	2.0	2.5	3.0	4.0
Mg^{2+} (25mM)	2 µl	3 µl	4 µl	5 µl	6 µl	8 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 of 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	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1-3 minutes
Final Extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and 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

- The optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, template DNA, Mg^{2+}) depend on the template-primer pair and must be determined individually. It is especially important to titrate the Mg^{2+} concentration and the amount of enzyme required per assay. The standard concentration of Mg^{2+} is 2mM and amount of Pfu DNA Polymerase is 1.25U per 50 µl of reaction mixture.
- Pfu DNA Polymerase remains 95% active after 2 hours incubation at 95°C.
- The error rate of Pfu DNA Polymerase in PCR is 2.6×10^{-6} errors per nt per cycle; the accuracy (an inverse of error rate), an average number of correct nucleotides incorporated before making an error is 3.8×10^{-5} (determined according to the modified method described in)
- Pfu DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The enzyme has no detectable reverse transcriptase activity.
- Do not use dUTP in PCR.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Guidelines for preventing contamination of PCR reaction

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 10U Pfu DNA Polymerase 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 10U Pfu DNA Polymerase 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 10U Pfu DNA Polymerase with 1μg E.coli [3H]-RNA (40000cpm/μg) for 4 hours at 37°C and 70°C.