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Pfu DNA Polymerase

Product No.: 102-9012-90-2

Description

Pfu DNA polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has superior thermostability and proofreading properties compared to another thermostable polymerase. Its molecular weight is 90 kDa； PCR products are blunt-ended without 3'end dA； *Pfu* DNA Polymerase also possesses 3′→5′ exonuclease proofreading activity (high fidelity) that enables the polymerase to correct nucleotide-misincorporation errors, but no 5'→3' exonuclease activity.

Unlike Taq DNA polymerase ex: AmpliTaq or KlenTaq, the specific is determined by itself. Some other DNA Polymerase can be used with *Pfu*, and it can promote the amplification efficiency and correct nucleotide mis-incorporation error but use *Pfu* only will achieve higher fidelity.

Concentration: 5U/μL, 100 μL

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10nmol of isotopically labelled 4X dNTP into DE81-insoluble material in 30 minutes at 72°C.

Applications

High fidelity PCR
Gene high-fidelity clone, amplification and expression
Blunt-end PCR cloning
Gene Site-directed Mutagenesis

10 X PCR Reaction Buffer

100 mM KCl, 160 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 200mM Tris-HCl (pH8.8), 1% Triton X-100, 1 mg/ml BSA

Quality control

Nuclease-free; DNA-free; Purity>95% by SDS-PAGE; Can amplify 1kg and 2kb gene from 100ng lambda DNA.

Storage condition

Store at 50 mM Tris-HCl, pH8.2, 0.1 mM EDTA, 0.1% Tween 20, 0.1% NP-40, 1 mM DTT and 50% glycerol，-20°C for at least one year.

Main technical parameters

Extends at approximately 600bp/min； Optimum temperature is 65-75℃； dNTP working concentration is 100-300mM, Mg2+ Optimum concentration is 2-3 mM, Optimum pH is 8.1-9.1.

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Recommended Usage

Almost the same as Taq DNA Polymerase. In PCR， Quantity for 50 μL of reaction mixture require about 2.5U *Pfu* DNA Polymerase.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Keep solutions on ice.
3. Add to a thin-walled PCR tube, on ice.

Suggested PCR Reaction Mix (50 μL)

Reagent	Volume	Final concentration
10X *Pfu* PCR Buffer with Mg_2	5 μL	1 X
Forward Primer (10 μM)	1 μL	0.2 μM
Reverse Primer (10 μM)	1 μL	0.2 μM
dNTP mixture (10 mM)	1 μL	0.2 mM
Template DNA (1ng/ μL)	1 μL	0.02 ng/ μL
Pfu DNA Polymerase (5U / μL)	0.5 μL	2.5 Units
Autoclaved, distilled water	Up to 50 μL	

4. Gently vortex the sample and briefly centrifuge to collect all drops from walls of tube.
5. If using a thermal cycler without a heated lid, overlay the sample with a half volume of mineral oil or add an appropriate amount of wax.
6. Place samples in a thermal cycler preheated to 94°C and start PCR.

Thermocycling conditions for PCR

Cycling step	Temperature	Time
Initial denaturation	94℃	2 min
Denaturation	94℃	30 sec
Annealing* } 30cycles	50-68℃	50 sec
Extension	72℃	1 min /600bp
Post elongation	72℃	10 min
Hold	4℃	Indefinitely

*Anneal at Tm of primer ±2℃

Note: For optimal specificity and amplification rate the temperature and cycling times should be optimized for each new target or primer pair.

7. The sample can be stored at -20℃until use.

Notes:

1. Amplification efficiency is a little weaker than Taq DNA Polymerase. It is caused by its 3´-5´ exonuclease activity (high fidelity). When the DNA amplified is below 1.5kb, there is no obvious difference between Taq and *Pfu*.
2. Mg2+ is added in the 10×*Pfu* PCR Buffer already， and it can ensure high fidelity. Rising the PH value and Mg2+ can partly improve the amplification efficiency but will weak the fidelity. PCR Buffer for Taq DNA Polymerase is not recommended for *Pfu*.
3. When use *Pfu* for amplification high purity primers is required (Length: more than 18 bases. Tm: 55-80℃. Concentration: 0.1-0.5 μM). Its 3´→5´ exonuclease activity (high fidelity) may cause primer degradation, especially when there is no dNTP in the solution. So *Pfu* must be added just few minutes before PCR reaction.

4. For GC-rich template, *Pfu* will not weaken its activity when the denaturation temperature is 98 because of its superior thermostability. *Pfu* DNA polymerase in your PCR reactions results in blunt-ended PCR products.
5. T/A clone will not work.
6. dUTP, dITP and primers containing these nucleotides should not be used in PCR with *Pfu* DNA Polymerase because the binding of this enzyme to DNA templates with uracil and hypoxanthine stalls DNA synthesis.

Troubleshooting

Symptoms	Suggestions
No product or low yield	Allow an extension time of at least 1-2 minutes/ kb of PCR target.
	Optimize annealing temperature by lowering it in 5°C increments.
	Ensure that the appropriate buffer is used
	Add Pfu DNA polymerase last to the reaction mixture to minimize any potential primer degradation.
	To minimize the effects of high-GC content or secondary structure use higher denaturing temperatures (94–98°C), or use cosolvents such as DMSO in a 1-10% (v/v) final concentration or glycerol in a 5–20% (v/v) final concentration.
	Ensure that primer concentration is not too low. Use the recommended primer concentrations between 0.1 and 0.5 uM (generally 100–250 ng for typical 18-to 25-mer oligonucleotide primers in a 100-ul reaction volume).
	Optimize primers with respect to melting temperature, purity, GC content, and length.
	Optimize the Mg2+ concentration. Increase the total Mg2+ concentration to 2 mM and taking the concentrations of the dNTPs, primers, and EDTA into account.
	Increase the amount of Pfu DNA polymerase.
	Optimize denaturation time. Denaturation times of 30–60 seconds at 94-95°C are usually enough while longer denaturation times may damage the DNA template; use the shortest denaturation time compatible with successful PCR on the thermal cycler.
	Ensure that template used is intact and of high purity. Use template at an adequate concentration.
Multiple bands	Optimize primer annealing temperature by increasing the annealing temperature in 5°C increments and/or use a hot start.
	Check for nonspecific primer-template annealing. Use Perfect Match PCR enhancer to improve PCR product specificity.
Artifactual smears	Decrease the amount of *Pfu* DNA polymerase.
	Ensure that extension time is not too long.