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i-Pfu DNA Polymerase

Cat. No. INT-25181 250 Units

DESCRIPTION

i-Pfu DNA polymerase is a thermostable DNA polymerase purified from an *E.coli* strain carrying a plasmid with the cloned gene encoding *Pyrococcus furiosus* DNA polymerase. The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'=>3' direction in the presence of Mg^{2+} at 70-80°C. *Pfu* DNA Polymerase exhibits 3'=>5' exonuclease (proofreading) activity, but has no detectable 5'=>3' exonuclease activity.

Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied. For routine PCR, where simple detection of an amplification product or estimation of the product's size is important, Taq DNA polymerase is the obvious enzyme to choose. However, when the amplified product is to be cloned, expressed or used in mutagenesis studies, Pfu DNA Polymerase is a much better enzyme of choice for PCR. Pfu DNA Polymerase is also used in blends with Taq DNA polymerase, or amino-terminally truncated versions of Taq DNA polymerase, to amplify longer stretches of DNA in PCR with greater accuracy than Taq DNA polymerase alone.

STORAGE

Store at -20℃, and then stable for at least one year.

CHARACTERISTICS

- High Fidelity : presence of 3'→5' exonuclease (proofreading)
- Low Error : the lowest error rate of any thermostable DNA polymerase studied.
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.

APPLICATIONS

- Amplification of genomic DNA and cDNA targets up to 10kb long with high fidelity.
- Cloning with blunt ends

KIT CONTENTS

- *i*-PfuDNA Polymerase (2.5 units/ μl)	250 units
- 10x PCR Buffer (15mM Mg^{2+})	1ml
- 10x Mg^{2+} free Buffer	1ml
- 10mM dNTPs (2.5mM each)	500 μl
- 25mM Mg^{2+}	1ml

10x PCR BUFFER

- 300mM Tris-HCl (pH9.0)
- 200mM Salts consisting of Na^+ and NH_4^{2+}
- 20mM Mg^{2+}

TECHNICAL TIPS

General Reaction Mixture for PCR (total 50 μl)

Template	1ng-1μg
Primer 1	5-10 pmoles
Primer 2	5-10 pmoles
i-Pfu DNA Polymerase (2.5u/μl)	0.5-1μl
10x PCR buffer	5μl
dNTP Mixture (2.5mM each)	4μl
Sterilized distilled water	up to 50μl

Suggested Cycling Parameters

PCR cycle		Temp.	PCR product size	
			≤2kb	≥2kb
Initial denaturation		94℃	2min	2min
30-40 Cycles	Denaturation	94℃	20sec	20sec
	Annealing	50-65℃	10sec	10sec
	Extension	65-72℃	30sec ~1min/kb	1min 30sec ~2min/kb
Final extension		72 ℃	Optional. Normally, 2-5min	

Trouble Shooting

Observation	Solutions
No product or low yield	Increase extension time to 2 minutes per kb
	Use the recommended amount of DNA template
	Lower the annealing temperature in 2℃ increments
	Ensure that 10x i-Pfu reaction buffer is used
	Use the recommended primer concentrations
	Check the melting temperature, purity, GC content, and length of the primers
Multiple bands	Increase the annealing temperature in 2℃ intervals
Artifactual smears	Decrease the amount of i-Pfu DNA polymerase
	Reduce the extension time utilized

EXPERIMENTAL INFORMATION

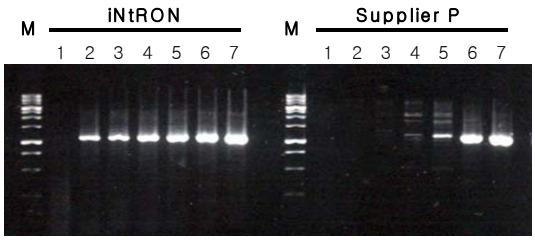

Fig. 1. Comparison of amplification sensitivity of iNtRON's recombinant *Pfu* DNA Polymerase to that of supplier P.
Human IL-10 gene (1.1kb) was amplified using iNtRON's (Panel A) or supplier's polymerase (Panel B) from genomic DNA of human stomach cancer cell line, AGS). After 40cycles of amplification, 5㎕ aliquots of the 25㎕ amplification reactions were analyzed on 1% agarose gel. The amounts of human genomic DNA template were: lane 1, no template; lane 2, 1.5ng; lane 3, 3ng; lane 4, 6ng; lane 5, 12ng; lane 6, 50ng; lane 7, 200ng; lane M, iNtRON's 1kb DNA Ladder (Cat. No. 24041)

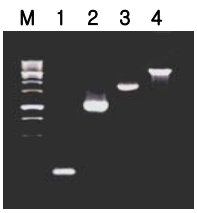

Fig. 2. Amplification of variable size of DNA fragments
DNA fragments were amplified with Pfu DNA polymerase lane 1, 200bp product; lane 2, 1.1kb product; lane 3, 1.8kb product ; lane 4, 4.5kb product ; lane M, 1kb DNA ladder (Cat. No. 24041)