For research use only

ISO9001

Pfu DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
Pfu DNA Polymerase	500 unit	EBT-1011	5 unit/μl
	500 unit	EBT-1012	with 1 ml dNTP mix

Description

Recombinant Pfu DNA polymerase is 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 (proof-reading) activity, but has no detectable 5′→3′ exonuclease activity.

The amplified products by Pfu DNA polymerase can be used for a gene cloning with decreased error rate, and for a site-specific mutagenesis. Pfu DNA Polymerase, like any other polymerases showing proof-reading activity, generates PCR products with blunt end.

Pfu DNA Polymerase is recommended for an amplification of DNA fragment smaller than 7 kbp. Pfu DNA Polymerase is provided with 10x optimized reaction buffer.

Storage Buffer

5 unit/μl in 50 mM Tris-HCl, pH8.2, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxynucleotides into a polynucleotide fraction in 30 min at 72°C.

10x Reaction Buffer

200 mM Tris-HCl, pH9.0, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1% Triton X-100, 1 mg/ml BSA.

QC tests

Activity, SDS-PAGE purity, performance tests, genomic DNA contamination test, confirmation test for the absence of endo and exonucleases.

Storage Condition

Store at -20°C.

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Standard Protocol

1. Prepare 50 μl PCR solution as follows:

PCR grade distilled water :	- μl
10x reaction buffer :	5 μl
10 mM dNTP mix (2.5 mM each) :	4 μl
Primer (10 pmol/μl) :	1 μl each
Template :	1-10 ng
Pfu DNA Polymerase :	0.5 μl (2.5 unit)

Adjust final vol. to 50 μl with PCR grade distilled water

*Note : Always, Pfu DNA polymerase should be added last to the mixture

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		<1 kbp	1-7 kpb
Denature	95°C	30 sec	30 sec
Anneal	Tm-4°C	30 sec	30 sec
Extend	72°C	45 sec	30-60 sec/1 kbp

25-40 PCR cycles

Trouble-Shooting

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
   - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragments.
2. Smear bands or smeared background
   - Reduce template concentration : High concentration of template can lead to smearing of PCR products. Generally, 1-10 ng of plasmid DNA is working well
   - Increase annealing temperature
3. Non-specific bands
   - Increase annealing temperature
   - Consider using PCR additives, like 1-2% DMSO and 0.5-1x Q buffer
   - Confirm specificity of your primers
4. Low yield
   - Increase enzyme concentration in the reaction
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added
5. Mutation is found
   - Increase initial template concentration
   - Reduce PCR cycle number
   - Reduce dNTP concentration added in PCR mix