For research use only

ISO9001

Pfu Super DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
Pfu Super DNA Polymerase	250 unit	EBT-1421	5 unit/μl
	500 unit	EBT-1422	5 unit/μl

Description

Pfu Super DNA Polymerase is suitable for a reliable amplification of long (up to 15 kbp) and complex targets with a robust yield and high accuracy. The error rate of Pfu Super DNA Polymerase is two times more less than that of Pfu DNA polymerase. The amplification efficiency (final yield) is about 2-3 times more than that of Pfu DNA polymerase. Furthermore, due to its high processivity, shorter running time is required compared to any other commercially available Pfu (5 kbp within 60 sec extension).

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

Pfu Super DNA Polymerase exhibits 3′→5′ exonuclease (proof-reading) activity, but has no detectable 5′→3′ exonuclease activity.

Pfu Super 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, pH 9.0, 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 Pfu Super buffer :	5 μl
10 mM dNTP mix (2.5 mM each) :	4 μl
Primer (10 pmol/μl) :	1 μl each
Template :	0.1-10 ng
Pfu Super Polymerase :	0.2-0.5 μl (1-2.5 unit)

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

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

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		1-2 kbp	3-5 kpb	> 6kbp
Denature	95°C	10 sec	20 sec	30 sec
Anneal	Tm-4°C	10 sec	20 sec	30 sec
Extend	72°C	20 sec	60 sec	20 sec/1 kbp

25-35 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 and 10-100 ng of genomic DNA are working well.
   - Reduce enzyme concentration in the reaction
   - Increase annealing temperature
   - Set up a reaction mix on ice
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