User Manual

Version 7.1

Product name: *Pfu* DNA Polymerase

Cat #: AD-100, AD-200, AD-OEM

Description:

Pfu DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum *Pyrococcus furiosus*. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'->3' direction. *Pfu* DNA Polymerase also exhibits 3'->5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5'->3' exonuclease activity. The main difference between *Pfu* and alternative enzymes is the *Pfu*'s superior thermostability and 'proofreading' properties. Unlike *Taq* DNA polymerase, *Pfu* DNA polymerase also possesses 3'->5' exonuclease proofreading activity, resulting in PCR fragments with fewer errors than *Taq*-generated PCR inserts. *Pfu* DNA polymerase is efficient for techniques that require high-fidelity DNA synthesis, but can also be used in conjunction with *Taq* polymerase to obtain the fidelity of *Pfu* with the speed of *Taq* polymerase activity.

Protocol:

	25 µl Reaction	**50 µl Reaction**	**Final Concentration**
10X Pfu reaction buffer	2.5 µl	5 µl	1X (see notes)
10 µM Primer A	1 µl	2 µl	400 µM
10 µM Primer B	1 µl	2 µl	400 µM
Template DNA	as needed	as needed	see notes
$MgCl_2$ (optional)	as needed	as needed	see notes
Pfu DNA polymerase (2.5U/µl)	0.5 µl	1 µl	see notes
Water (ddH_2O)	up to 25 µl	up to 50 µl	

Thermocycling Conditions

3 Step PCR

Step	**Temperature**	**Time**	
Initial Denaturation	95°C	2-5 minutes	25-35 cycles
Denaturation	94°C	30 seconds	
Annealing	45°C - 68°C **	30 seconds	
Extension	72°C	30 - 60 seconds / kb	
Final Extension	72°C	1-5 minutes	
	4°C	Hold	

** 3-5°C lower than lowest tm primer

Notes

For more robust amplification, add addition Pfu DNA polymerase as needed in 0.5 µl increments.

Recommended DNA Template addition

- Genomic DNA 50-250 ng
- Plasmid DNA 1pg-10ng
- Viral DNA 1pg-10ng

Mg_2^+

The final concentration of Mg_2^+ in 1X Pfu reaction buffer is 2 mM. Add additional Mg_2^+ as needed in 0.5 mM increments.
Suggested final Mg_2^+ concentration ranges from 2 mM to 4 mM.