Gene to Protein Pvt. Ltd.
www.genetoprotein.com
info@genetoprotein.com
800 GENOME, 800 GENETIC

Pfu DNA Polymerase

Cat # ☐ **PCR003** ☐ **PCR004** ☐ **PCR003-S**
Pack Size: ☐ **250U** ☐ **1000U** ☐ **100U recommended to use 1.25 U per reaction of 25 ul**
Storage and Handling:

- Store at -20°C in a frost-free freezer. Pfu DNA Polymerase is stable for up to 1 years if stored at -20°C. Avoid repeated freeze-thaw cycles. Prior to use, thaw the reaction Buffer on ice. After opening, store at -20°C

Kit contents:

Componenets	PCR003	PCR004	PCR003-S
PFU Polymerase 5U/µL	50 µL	200 µL	20 µL
10X Reaction Buffer	1mL	3mL	1mL

Introduction

Pfu DNA polymerase was discovered in *Pyrococcus furiosus*. The polymerase enzyme catalyses the polymerization of nucleotides in the 5'-to-3' direction at a temperature of 75 °C in the presence of Mg^{+2}. Additionally, Pfu DNA Polymerase has 3'–5' exonuclease (proofreading) activity. During this proofreading process, any base misincorporations that may happen during polymerization are quickly removed. Since high-fidelity synthesis is required for PCR and primer extension reactions, Pfu DNA Polymerase is advised for use in these processes. The PCR fragments produced by Pfu DNA Polymerase have blunt ends.

10X Reaction Buffer with $MgCl_2$: The reaction buffer has been optimized to work with wide range of template. It contains, Tris-HCl (pH 8.8 at 25°C), KCl, 20mM $MgCl_2$ and Triton® X-100.

Note: 10X reaction buffer can be supplied without $MgCl_2$ on demand. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Source: *Pyrococcus furiosus* strain

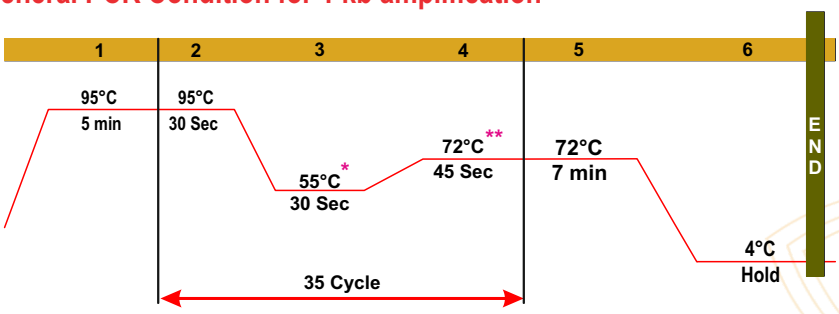
Storage Buffer: 50mM Tris-HCl (pH 8.0 at 25°C), 0.1mM EDTA, 2mM DTT, 50% glycerol.

Unit: One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 72°C

Standard PCR Reaction mix

Component	For 25ul reaction	For 50ul reaction	Final Concentration
Template (DNA)	Variable	Variable	<1,000 ng
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
10 mM dNTP each	0.5 µl	1 µl	200µM
10X Reaction Buffer	2.5 µl	5 µl	1X
Pfu DNA Polymerase	0.25 µl	0.5 µl	1.25 units /25µl PCR reaction
Nuclease-Free Water	to 25 µl	to 50 µl	

General PCR Condition for 1 kb amplification



* Depends on the primer Tm and template used (Need to be optimized)
** Allow approximately 1 minutes for every 1kb to be amplified.

Result

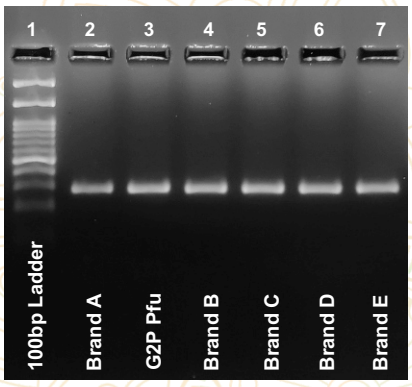


Fig: Gene was amplified and loaded on 2% agarose gel. Lane 3 represent the amplification using Pfu DNA Polymerase from Gene to protein Pvt Ltd.

COMPONENTS OF THE REACTION MIXTURE

Template DNA; For both plasmid and phage DNA, the ideal amounts of template DNA in the 50-µL reaction volume are ***0.01–1 ng***, and for genomic DNA, they are 0.1–1 µg. greater number of templates raises the possibility of non-specific PCR product production. The accuracy of the amplification is decreased with less template. The preparation of templates can be done using any standard DNA purification technique, such as G2P Genomic DNA Purification Kits (PUR11/12/13/14/15/16/17/18/19/20). Taq DNA polymerases can be inhibited by traces of some chemicals used for DNA purification, including phenol, EDTA, and proteinase K. Trace impurities are often removed from DNA samples using ethanol precipitation and repeated washing with 70% ethanol on the DNA pellet.

Concentration of $MgCl_2$; Because Mg^{2+} binds to dNTPs, primers, and DNA templates, Mg^{2+} concentration must be adjusted. The concentration range indicated is 1-4 mM. The yield of the PCR product may be lowered if the Mg^{2+} content is too low. If the Mg^{2+} concentration is too high, non-specific PCR products may arise and PCR fidelity may be lowered. Taq DNA Polymerase is supplied with ***20mM of $MgCl_2$*** which is optimal in most of the case.

dNTPs; Each dNTP should be used at a final concentration of 0.2 mM. Higher dNTP concentrations are preferred in certain PCR applications. Because Mg^{2+} binds to dNTPs, the $MgCl_2$ concentration must be adjusted correspondingly. The reaction mixture must contain equal amounts of all four nucleotides (dATP, dCTP, dGTP, and dTTP). Use the following dNTP mix volumes to achieve a ***0.2 mM concentration of each dNTP*** in the PCR mixture.

Primers; The PCR primers' suggested concentration range is***0.1-1 µM*** Excessive primer concentrations raise mispriming and the production of non-specific PCR products are both possible. Higher primer concentrations in the region of 0.3-1 M are frequently preferred for defective primers.

Troubleshooting

	Possible Cause	Recommendation
Little or no PCR Product	Difficult template e.g., GC-rich templates	• Add DMSO (final concentration, 8%) and reduce enzyme concentration (e.g., use as little as 0.5 U per reaction).
	DNA template problems	Check quality and concentration of template: • Analyze an aliquot on an agarose gel to check for possible degradation. • Test the template with an established primer pair or PCR system. • Check or repeat template purification.
	Enzyme concentration too low	• Increase enzyme concentration to 2 U Taq DNA Polymerase per 50 µl reaction.
	$MgCl_2$concentration too low	Increase the $MgCl_2$concentration in 0.25 mM steps; the minimal acceptable concentration is 1.5 mM $MgCl_2$
	Cycle conditions not optimal	• Decrease annealing temperature. • Increase cycle number. • Make sure that the final elongation step is included in the program.
	Primer design not optimal	Design alternative primers.
	Primer concentration not optimal	• Both primers must have the same concentration. • Titrate primer concentration (0.1 to 0.6 M).
	Primer quality or storage problems	• If you use an established primer pair, check performance in an established PCR system (e.g., with a control template). • Make sure that the primers are not degraded. • Always store primers at –15 to –25°C.
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature (Longer primers have higher annealing temperatures).
	Primer design or concentration not optimal	• Review primer design. • Titrate primer concentration (0.1 to 0.6 M). • Both primers must have the same concentration. • Perform nested PCR with nested primers.
	DNA template problems	Use serial dilution of template.
PCR products in negative control experiments	Carryover contamination	• Replace all reagents, especially water. • Use aerosol-resistant pipette tips. • Set up PCR reactions in an area separate from that used for PCR product analysis. • To eliminate carryover contaminants: Use dUTP* (600mM) instead of dTTP (200 M) and thermolabile UNG* (1 U/50 lreaction); also, increase Mg2+concentration (to a maximum of 4 mM) to compensate for higher dNTP conc