Pfu DNA polymerase

Cat: PE2010 **Size: 500 Units**
Conc. 5 units/µl Store at -20°C.

Description
Pfu DNA Polymerase is a thermostable enzyme with a molecular weight of 90 kDa. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction, resulting in blunt-ended PCR products without 3'-dA overhangs. Pfu DNA Polymerase exhibits 3'→5' exonuclease (proofreading) activity that enables the polymerase to correct the mis-incorporation of nucleotide, and lacks 5'→3' exonuclease activity. It is suitable for PCR and primer extension reaction that requires high fidelity when the PCR fragment is relatively shorter than 3kb.
The extension rate of Pfu DNA Polymerase is about 600bp/min in standard condition. The appropriate reaction temperature is 65ºC~75ºC, the working concentration of dNTPs is 100-300µM, the working concentration of Mg^{2+} is 2~3mM, and the optimal pH is 8.1~9.1. The amount of enzyme is 1~1.5unit for 20µl PCR reaction, while 2~3units for 50µl PCR reaction.

Reaction Buffer (PCR Buffer, 10X):
100mM KCl, 160mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 200mM Tris-HCl (pH8.8), 1% Triton X-100, 1mg/ml BSA

Storage Buffer
Pfu DNA Polymerase is supplied in 50 mM Tris-HCl (pH 8.2), 0.1 mM EDTA, 0.1% Tween20, 0.1% NP-40, 1mM DTT, and 50% Glycerol.

Unit Definition
One unit incorporates 10 nanomole of dNTPs into acid insoluble material in 30 minutes at 74 ºC

Product Contents:

1. Pfu DNA Polymerase (5 units/ul)	100 ul (500 U)
2. 10 x PCR reaction buffer	1.5 ml

Protocol

1- In a 0.2 ml or 0.5 ml thin wall tube, add the following components:

	20 ul reaction	50 ul reaction
10 x PCR reaction buffer	2 ul	5 ul
Forward primer	10~20 pmol	20~50 pmol
Reverse primer	10~20 pmol	20~50 pmol
Template DNA	5~50 ng	10~100 ng
dNTPs (10 mM each)	0.5 ul	1 ul
PCR dye (optional)	2 ul	5 ul
Pfu DNA Polymerase	1-1.5u	2-3u
ddH_2O	X ul	X ul
Total	20 ul	50 ul

2- Mix gently by pipetting, close the tube and centrifuge for a few seconds.
3- Add mineral oil to each tube (this step is unnecessary when using a thermal cycler with top heating).
4- Perform PCR cycles according to the PCR condition. An example is given as follows:
(Annealing temperature and time need to be optimized for each primer/template combination).

94 °C	2.5 min	
94 °C	45 sec	} 25~35 cycles
50~72 °C	1 min	
72 °C	1~3 min	
72 °C	5~10 min	

5- Run 2~5 ul PCR products on 1% agarose gel stained with GelRed, GelGreen or Et.Br

Accessory Products

The following products are available separately from Genbiotech.

Product	Quantity	Catalog no.
Taq DNA polymerase	500 U	PE1010
dNTPs in Separate Tube (100 mM each)	4 x 100 ul	PD2001
dNTPs in Separate Tube (100 mM each)	4 x 250 ul	PD2002
dNTPs in Separate Tube (100 mM each)	4 x 400 ul	PD2003
EvaGreen™, 20X in water	5 x 1 ml	3100
Loading Dye 6X	3 x 1 ml.	NM4011
Custom oligonucleotides		