Pfu DNA polymerase

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 enzyme exhibits 3´>5´ proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Buffers and Reagents:

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20,0.5 % Nonidet P-40, 50% Glycerol.

10X Reaction Buffer: Contains Tris-HCl (pH 9.0), PCR enhancers,KCl, (NH4)2SO4

Contents:

Components	C101141	C101142
Pfu DNA p. 5 U/µl	**250 U**	**500 U**
$MgCl_2$ Solution 25 mM	**0.5 ml**	**1 ml**
10X Buffer $MgCl_2$ free	**0.5 ml**	**1 ml**

Kit storage: This kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for one year from the date of production.

General Reaction Protocol:

Please be sure to follow below

1. Thaw 10X reaction buffer , dNTP mixture.

Component	Vol (µL)
10X Reaction Buffer	2 µL
MgCl2 Solution 25mM	1.2 µL
40 mM dNTPs Mix (10 mM each)	0.5 µL
Upstream Primer (10 pmol/µl)	1 µL
Downstream Primer(10 pmol/µl)	1 µL
***Pfu DNA poly.* (5 units/µL)**	0.25 µL
Template DNA	Variable
Sterilized D.W.	Variable
Total Volume	20 µL

2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
3. Add templates DNA to the individual PCR tubes or wells containing the master mix.
5. Program the PCR machine according to the program outlined.

6. Place the PCR tubes or PCR plates in the terminal cycler and start the cycling program.

7. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

* Longer extension time makes nonspecific bands

Cycle	Time	Temp °C
1	3 Min	94
25 ~35	30 Sec	94
	30 Sec	50~60
	30 Sec	72*
1	3 Min	72

*Extension rate for this enzyme is near 1000 bp/min.