KIAGENE FANAVAR

Pfu DNA polymerase

Cat. No:
FPLF002.0250
FPLF002.0500

Contents:

Components	100 RXN	200 RXN
Pfu DNA poly. 5 U/µl	250 U	500 U
MgCl2 Solution 25 mM	0.5 ml	1 ml
5X Pfu Buffer MgCl2 free	0.5 ml	1 ml

Kit storage:

This kit should be stored at -20 ºC. Under this condition reagents are stable for two years from the date of production.

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

Protocol:

1) Thaw 5X reaction buffer, dNTP mixture.
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.

Component	Volume	Final Conc.
5X Reaction Buffer	4 µl	1X
MgCl2 Solution 25 mM	1.6 µl	2 mM
40 mM dNTPs Mix (10 mM each)	0.4 µl	0.2 mM
Upstream Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µl
Downstream Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µl
Template DNA	Variable	10 fg~1 µg
PCR grade water	Variable	-
Pfu DNA poly. (5 units/µl)	0.25 µl	-
Total Volume	**20µl**	-

4) Program the PCR machine according to the program outlined.

Cycle	Time	Temp °C
1	4 min	95
30-35	30 sec 30 sec 60 sec	94 57 72
1	5 min	72

Note:

* Longer extension time makes nonspecific bands
* Extension rate for this enzyme is near 500bp/min.

Agarose gel Electrophoresis:

Run the total 5-7 µL of PCR products alongside 3 µL DNA marker on a 2% agarose gel containing DNA safe stain.

Disclaimers and Addresses:

This product is for **Research use only** and should only be used by trained professionals.