KIAGENE FANAVAR

Phusion DNA polymerase

Cat. No:

FPLF004.0250

FPLF004.0500

Contents:

Component	100RXN	200RXN
Phusion DNA poly. 5 U/µl	50 µl	100 µl
MgCl2 Solution 25 mM	500 µl	1 ml
5X Phusion Buffer $MgCl_2$ free	500 µl	1 ml

Description:

Phusion DNA Polymerase is a chimeric Pfu which has a DNA binding protein at the N-terminal portion of the gene. This enzyme keeps significant activity after exposure to 99 °C or repeated exposure to 98 °C with more processivity and extention rate than Pfu DNA polymerase. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'_3' direction, resulting in blunt-ended PCR products **without 3'-dA overhangs.**

Phusion 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 **higher than 3 kb.** The enzyme exhibits 3´>5´ proofreading activity,

resulting in over 20-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Kit storage:

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

Component	Volume	Final Conc.
5X Reaction Buffer	4 µL	1X
MgCl2 Solution 25 mM	1.2 µL	1.5 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	-
Phusion DNA poly. 5 U/µl	0.25 µl	-
Total Volume	20µl	-

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.
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 3000 bp/min.

Agarose gel Electrophoresis:

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