Product Information Sheet and Protocol

Product Name: PFU-50 High Fidelity Polymerase*
Concentration: 2.5 Units/µL
Ordering Information:

Item Number	Units	Enzyme Component Information
SS-PFU-50-20	20	PFU-50: 8uL, 1 tube at 20 Units/tube 10X PFU Reaction Buffer: 1 tube at 0.125mL/tube
PFU-50-100	100	PFU-50: 40uL, 1 tube at 100 Units/tube 10X PFU Reaction Buffer: 1 tube at 500uL/tube
PFU-50-200	200	PFU-50: 80uL, 2 tubes at 100 Units/tube and 40uL/tube 10X PFU Reaction Buffer: 2 tube at 500uL/tube
PFU-50-500	500	PFU-50: 200uL, 1 tube at 500 Units/tube 10X PFU Reaction Buffer: 2 tubes at 1.25mL/tube
PFU-50-1000	1000	PFU-50: 400uL, 2 tubes at 500 Units/tube and 200uL/tube 10X PFU Reaction Buffer: 4 tubes at 1.25mL/tube
PFU-50-5000	5000	PFU-50: 2000uL, 10 tubes at 500 Units/tube and 200uL/tube 10X PFU Reaction Buffer: 20 tubes at 1.25mL/tube

Storage and Handling:
Store at -20°C upon arrival.

Product Description:
Pfu-50 Polymerase is the ideal choice for applications where the efficient amplification of DNA with highest fidelity is required. The enzyme is a genetically engineered Pfu DNA polymerase, but showing a 2-fold higher accuracy and an increased processivity, resulting in shorter elongation times. The enzyme catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction but does not possess a 5'→3' exonuclease replacement activity. Its inherent 3'→5' exonuclease proofreading activity results in a greatly increased fidelity of DNA synthesis compared to Taq polymerase. Pfu-50 Polymerase-generated PCR fragments are blunt

Pfu-50 Polymerase is characterized by a 50-fold higher fidelity compared to Taq polymerase and a 2-fold higher fidelity compared to standard Pfu polymerase. ERPfu-50 Polymerase = 0.25×10^{-6} The error rate (ER) of a PCR reaction is calculated using the equation ER = MF/(bp x d), where MF is the mutation frequency, bp is the number of base pairs of the fragment and d is the number of doublings.

Components:
PFU-50 Pol: 2.5 units/µl PFU-50 Polymerase in storage buffer (50 % Glycerol, 50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT 0.1 % Tween 20, 0.1 % Nonidet P-40)
10X PFU-50 Buffer

* This product is for "Research Use Only. Not for use in diagnostic procedures".
For MSDS and Certificate of Analysis please visit www.empiricalbioscience.com

Recommended Protocol (50uL Reaction):

PCR Component	Volume/Concentration
10X PFU-50 Reaction Buffer	5µL
dNTP	200µM
Primers	0.4µM
Template DNA	1-100ng
PFU-50 High Fidelity Polymerase**	0.5µL (1.25 Units)
PCR Grade Water	Fill to 50uL

****Add polymerase as last component**

Recommended Cycling Conditions:

Three-step standard protocol

Cycling Step	Temperature	Holding Time	Cycles
Initial Denaturation	95°C	2 minutes	1
Denaturation	95°C	20 seconds	25-30
Annealing[1)]	50-68°C	30 seconds	
Elongation[2)]	68°C	1min/kb	
Final Elongation	68°C	1min/kb	1

[1)]The annealing temperature depends on the melting temperature of the primers used.
[2)]The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

Two-step protocol for amplification of longer fragments (>3kb)

Cycling Step	Temperature	Holding Time	Cycles
Initial Denaturation	95°C	2 minutes	1
Denaturation	95°C	20 seconds	25-30
Annealing[1)]/ Elongation[2)]	68°C	30 sec/kb	
Final Elongation	68°C	30 sec/kb	1

[1)]The annealing temperature depends on the melting temperature of the primers used.
[2)]The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.