Product Information Sheet

Product Name: Phu-50 High Fidelity Polymerase with 10X Phu Reaction Buffer*

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Concentration:

Phu-50 High Fidelity Polymerase: 2.5 Units/µL
Phu-50 Reaction Buffer: 10X

Storage and Handling:

Upon arrival store at -20°C for provided expiration date. Minimize Freeze thaw to avoid loss of performance.

Product Description:

Phu-50 High Fidelity Polymerase:

Is the ideal choice for applications where the efficient amplification of DNA with highest fidelity is required. The enzyme is a genetically engineered Phu 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. Phu-50 Polymerase-generated PCR fragments are blunt

Phu-50 Polymerase is characterized by a 50-fold higher fidelity compared to Taq polymerase and a 2-fold higher fidelity compared to standard Phu polymerase. ER Phu-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.

Empirical Phu-50 High Fidelity Polymerase is supplied in a storage buffer containing: Glycerol, Tris-HCl, EDTA, DTT, Tween 20, and Nonidet P-40.

10X Phu-50 Reaction Buffer:

Specially optimized reaction buffer to be used with Phu-50 Polymerase. This Buffer is supplied in a 10X concentration and should be diluted for use.

Protocol:

The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

PCR Component	Volume	Final Concentration
10X PHU-50 Reaction Buffer	5µL	1X
dNTP, 10mM	2µL	400µM
Primers, 10µM	1µL	0.2µM
DNA Template	XµL	1-100ng
Phu-50 High Fidelity Polymerase**	0.5µL	0.5µL (1.25 Units)
PCR Grade Water	Fill to 50uL	Not Applicable

****Add polymerase as last component**

***This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.**

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Thermal cycling conditions: The following general cycling conditions are recommended but can vary depending on the template and primers being used.

Three-step protocol

Cycling Step	Temperature	Holding Time	Cycles
Initial Denaturation	95°C	1-2 minutes	1
Denaturation	95°C	15 seconds	25-30
Annealing[1)]	50-68°C	20-30 seconds	
Elongation[2)]	68-72°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.

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