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Product Information Sheet

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

Concentration: Phu-50 High Fidelity Polymerase: 2.5 Units/µL, Phu Reaction Buffer: 10X
Storage and Handling: Upon arrival store at -20°C for provided expiration date
Ordering Information:

Item Number	Units	Enzyme Component Information
PHU-50-250	250	Phu-50 High Fidelity Polymerase: 1 x 100µL, 10X Phu Reaction Buffer: 1 x0.75mL
PHU-50-500	500	Phu-50 High Fidelity Polymerase: 2 x 100µL, 10X Phu Reaction Buffer: 4 x0.75mL
PHU-50-1000	1000	Phu-50 High Fidelity Polymerase: 4 x 100µL,10X Phu Reaction Buffer: 8 x0.75mL
PHU-50-5000	5000	Phu-50 High Fidelity Polymerase: 20 x 100µL,10X Phu Reaction Buffer: 40 x0.75mL

Product Description:
Phu-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 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 pH 8.0, 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: Minimize Freeze thaw to avoid loss of performance. 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**

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.