WizPure™ Pfu DNA Polymerase

RUO For Research Use Only

REF W1330

DESCRIPTION

WizPure™ Pfu DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum Pyrococcus furiosus. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction. Pfu DNA Polymerase also possesses 3'→5' exonuclease (proofreading) activity. WizPure™ Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied, is even up to ten fold more accurate than normal Taq DNA polymerase. Consequently, WizPure™ Pfu DNA Polymerase is useful for polymerization reactions requiring high-fidelity synthesis.

KIT CONTENTS

Contents	W1330	W1331	W1331-5
Pfu Polymerase (2.5U/µl)	100 µl	200 µl	1,000 µl
10X Pfu Buffer	1,000 µl	2,000 µl	8,000 µl
dNTP mix (2.5 mM each)	500 µl	1,000 µl	5,000 µl

APPLICATIONS

- High-fidelity PCR and primer-extension reactions
- Generation of PCR products for cloning and expression.
- PCR cloning and blunt-end amplification product generation
- RT-PCR for cDNA cloning and expression
- Site-directed mutagenesis
- Blunt-end PCR cloning

STORAGE BUFFER

20mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20, and 0.5% Nonidet P40.

10X PFU BUFFER

200mM Tris-HCl, 100mM KCl, 100mM (NH4)2SO4, 1% Triton X-100, 1mg/ml BSA, 20mM MgSO4, pH 8.8 (25°C).

UNIT DEFINITION

1 unit of the enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction in 30 min at 70°C.

STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer.

PROTOCOL

General PCR reaction mixture for 50 µl Reaction :

On ice, prepare each of following master mixes, combine, and place in heated (to 94°C) thermal cycler:

In a sterile, nuclease-free microcentrifuge tube, combine the following components:

For 50µl PCR Reaction	Volume	Final Conc.
Pfu Polymerase (2.5U/µl)	0.2 ~ 1 µl	0.5 ~ 2.5 U
10X PCR Buffer	5 µl	1 X
dNTP mix (2.5 mM each)	4 µl	200 µM each
Template	< 500 ng	< 500 ng
Forward Primer	5 ~ 50 pmol	0.1 ~ 1 µM
Reverse Primer	5 ~ 50 pmol	0.1 ~ 1 µM
Distilled water	up to 50 µl	

Recommended PCR Cycling Conditions :

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	1 ~ 5 min.	1
Denature	95	30 sec.~ 1min.	25 ~ 40
Anneal	50~65	30 sec.~ 1min.	
Extend	72	30 sec.~ 1min.	
Final Extension	72	5 min.	1

IMPORTANT: Annealing temperature should be 2-6°C lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

Notice :

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

QUALITY CONTROL ASSAY

Contamination Assay

WizPure™ Pfu DNA Polymerase was passed from quality control assay for contamination of bacterial host DNA using sequence-specific primer set from host bacterial genomic DNA.

Functional assay

WizPure™ Pfu DNA Polymerase was functionally tested for PCR amplifications using the various size from human genomic DNA.

ORDERING INFORMATION

Product	Cat No.	Package	Note
WizPure™ Pfu DNA Polymerase	W1330ES	250 U	-
	W1330E	500 U	-
	W1330E-5	2,500 U	-
	W1330S	250 U	dNTP
	W1330	500 U	dNTP
	W1330-5	2,500 U	dNTP

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