Pfu DNA Polymerase

Catalog #	Pack size	Price(€)
ZP00202	500U(5U/ul)	**14.40**
ZP00203	1000U(5U/ul)	**27.20**

Description:

Pfu DNA Polymerase is a thermostable enzyme of approximately 90kDa isolated from Pyrococcus furiosus. The enzyme replicates DNA at 75^{o}C, catalyzing the polymerization of nucleotides into duplex DNA in the 5′-3′ direction in the presence of magnesium. Pfu DNA Polymerase also possesses 3′-5′ exonuclease (proofreading) activity. Base misinsertions that may occur during polymerization are rapidly excised by the proofreading activity of the polymerase. Consequently, Pfu DNA Polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. Pfu DNA Polymerase-generated PCR fragments are blunt-ended. **Elongation rate is 2Kbp/min.**

Source:

Pyrococcus furiosus strain Vc1 DSM3638.

Features:

High Fidelity: Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase.

Pfu DNA Polymerase. extends at approximately **2K bp/min**

Applications:

Pfu DNA Polymerase is recommended for use in PCR, primer extension reactions and other applications that demand high fidelity.

Unit Definition:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10nmol of dNTPs into acid-insoluble material in 30 minutes at 75^{o}C.

Concentration:5U/ul

Reaction Mixture Set Up

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Keep solutions on ice.
3. Add to a thin-walled PCR tube, on ice:

Reagent	Quantity, for 50 µl of reaction mixture	Final concentration
5X *Pfu* buffer	10ul	1X
10mM dNTP	1ul	0.2mM
PrimerI(25pmol/ul)	1ul	0.5pmol/ul
PrimerII(25pmol/ul)	1ul	0.5pmol/ul
Pfu(5U/ul)	0.4ul	2U/50ul
Template DNA	variable	50 pg -1 µg
ddH2O	Up to 50ul	

4. Gently vortex the sample and briefly centrifuge to collect all drops from walls of tube.
5. If using a thermal cycler without a heated lid, overlay the sample with a half volume of mineral
oil or add an appropriate amount of wax.
6. Place samples in a thermal cycler preheated to 96°C and start PCR.

Recommended thermal cycling conditions:

Step	Temperature,°C	Time ,min	Number of cycles
Initial denaturation	96	4	1
Denaturation	95	0.5	
Annealing	50-68	0.5-2	25-35
Extension	72	0.5-4	2Kbp/min
Final Extension	72	5	1

Quality Control Tests:

PCR (activity), SDS-PAGE (purity), endonuclease/nickase.

Storage:

Pfu DNA Polymerase in 50mM Tris-HCl (pH 8.2 at 25^{o}C), 0.1mM EDTA, 1mM DTT, 50% glycerol and 0.05% CHAPS should be stored at -20^{o}C.

5X Reaction Buffer with $MgSO_4$:

100mM Tris-HCl (pH 8.8 at 25°C), 50mM KCl, 50mM $(NH_4)_2SO_4$, 10mM $MgSO_4$, 0.5% Triton X-100 and 0.5mg/ml nuclease-free BSA.

References:

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Lundberg, K.S. et al. (1991) *Gene* 108, 1-6.
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Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add：Floor 2,Building A,328#, Wuhe Road,Shanghai,201109,China

Tel：+86-21-54460832

Fax:+86-21-54460831

E-mail:master@shinegene.org.cn

Website：www.synthesisgene.com