Datasheet

Description:
Pfu/Psp DNA polymerase, isolated from the archae bacteria Pyrococcus furiosus/species is a thermostable Polymerase of approximately 90000 daltons. Base misinsertions that may occur during polymerization are rapidly excised by the proofreading activity of the polymerase. The Pfu DNA Polymerase has no detectable reverse transcriptase activity.

Features:
Pfu/Psp DNA polymerase replicates DNA at 75°C catalyzing the polymerization of nucleotides into duplex DNA in the 5´=>3´ direction in the presence of Mg^{+}. Pfu DNA polymerase possesses 3' to 5' exonuclease proof reading activity that enables the polymerase to correct nucleotide-misincorporation errors. The enzyme has no 5'=>3' exonuclease activity.

Storage: at -20°C for 24 months

Transportation: on blue ice

Applications:
- blunt end PCR cloning
- PCR and primer extension where "high fidelity" is required
- Site-directed mutagenesis

Concentration: 5 u/µl

Unit definition:
One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nM of dNTPs into acid insoluble material in 30 minutes at 75°C.

Storage Buffer:
50 mM Tris-HCl, pH 8.2, 0.1 mM EDTA, 0.1% Tween 20, 0.1% Nonident P40, 1 mM DTT, 50% Glycerol

Reaction Buffer 10 X:
100 mM KCl, 160 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 200 mM Tris-HCl, pH8.8, 1% Triton X-100, 1 mg/ml BSA

Quality control:
- Tested for the DNA amplification of 2,2 kb from lambda DNA
- Contamination level check of bacterial DNA
- Purity by SDS-Page > 90 %

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Usage:

Standard protocol:
- Do not use dUTP or dITP or primers containing these nucleotides

Components	Volume per reaction	end conc.
10X reaction buffer with $MgSO_4$	5 µl	1X
dNTP-Mix (40mM = 10mM each)	1.0 µl	200 µM each
Up-stream primer (e.g. 20 µM)	0,5 µl	0.1-1.0 µM
Down-stream primer (e.g. 20 µM)	0.5 µl	0.1-1.0 µM
Template DNA (10 ng/µl)	1.0 µl	<= 0,5 µg
Pfu/Psp DNA Polymerase (5 u/µl)	0.2 - 0,4 µl	1-2 units
Sterile dest. Water (molecular grade)	up to **50 µl**	

Note:
- vortex all solutions carefully before using
- dispense all reagents on ice to avoid degradation of primers and dNTP's
- add the enzyme after Template DNA
- may you have to optimize the $MgSO_4$ concentration for best result

General Thermo-Cycler protocol:

Step	Time	Temperature
Initial denaturation	1-3 min	95°C
25-35 Cycles: Denaturation Annealing Extension	 30-100 sec 30-65 sec 1-2 min (per 1kb)	 95°C 37-69°C 72-75°C
Final extension	5 min	72-75°C

Order Information

Prod. No.	Description	Quantity
S9116	Pfu/Psp DNA Polymerase	1 x 250 units
S9117	Pfu/Psp DNA Polymerase	2 x 250 units
S9118	Pfu/Psp DNA Polymerase	10 x 250 units