

Pfu/Psp red DNA Polymerase (ready-to-load)

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. For visual control and for fast loading on the gel the enzyme **contains a red dye and loading buffer for agarose electrophoresis.**

Applications:

- blunt end PCR cloning
- PCR and primer extension where "high fidelity" is required
- Site-directed mutagenesis
- PCR where visual control is needed

Description:

Pfu/Psp DNA polymerase **ready-to-load** is isolated from the archae bacteria Pyrococcus f-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/Psp DNA Polymerase has **no detectable reverse transcriptase activity.**

Concentration: (1 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 and red dye

Reaction Buffer 10 X:

100 mM KCl, 160 mM (NH₄)₂SO₄, 20 mM MgSO₄, 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 %

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 (1 u/μl)	1 - 2 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 MgSO₄ 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 1 kb)	95 °C 37-69 °C 72-75 °C
Final extension	5 min	72-75 °C

Loading on the gel:

Recommended volume is 10 µl of reaction mixture

Storage: at -20 °C for 24 months

Transportation: on blue ice

Related products:

Ordering Information:

Cat.-no	Description	Amount
S127	Pfu/Psp RTL DNA Polymerase	1x250 units
S119	Pfu/Psp RTL DNA Polymerase	2x250 units
S120	Pfu/Psp RTL DNA Polymerase	10x250 units