

## Pfu/Psp DNA Polymerase 2X-preMix

### 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<sup>+</sup>. Pfu DNA polymerase possesses 3' to 5' exonuclease proof reading activity that enables the polymerase to correct nucleotide-misincorporation errors. To reduce the risk of contamination, pipetting errors and to increase the repeatability of results the 2X-preMix contains an optimized mixture of enzyme, dNTP's and reaction buffer. Just add your template DNA and primers.

### 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 2X-preMix is isolated from the archae bacteria Pyrococcus f-species, 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:** Premix 2X (25µl per reaction)

### 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.

### 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.
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 2X-preMix	25 µl	1 unit
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 Mg<sup>+</sup> concentration for best result

### General Thermo-Cycler protocol:

Step	Time	Temperature
Initial denaturation	1-3 min	95 °C
<b>25-35 Cycles:</b>		
Denaturation	30-100 sec	95 °C
Annealing	30-65 sec	37-69 °C
	1-2 min (per 1kb)	72-75 °C

Extension		
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