Datasheet

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**.

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. To reduce the risk of contamination, pipetting errors and to increase the repeatable 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

Concentration: Premix 2X (25µl per reaction)

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

Storage: at -20°C for 24 months

Transportation: on blue ice

Stability tests / Quality Tests
- 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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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^{+} concentration for best result

General Thermo-Cycler protocol:

Step	Time	Temperature
Initial denaturation	1-3 min	95°C
25-35Cycles: Denaturation Annealing Extension	 30-10sec 30-65 sec 1-2 min (per 1kb)	 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

Order information

Prod. No.	Description	Quantity
S9121	Pfu DNA Polymerase 2X Pre-mix	100 rcs
S9122	Pfu DNA Polymerase 2X Pre-mix	5x 100 rcs

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