

The ENZYME Company

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### **Psp DNA polymerase (DNA-tested)**

**Description** *Psp* DNA polymerase, isolated from the hyperthermophilic archae bacteria *Pyrococcus species* is a thermostable Polymerase of approximately 92 kDa. The enzyme replicates DNA at 75°C, catalyzing the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium (prefers MgSO<sub>4</sub>). Unlike *Taq* DNA polymerase, *Psp* DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that *Psp* DNA polymerase-generated PCR fragments will exhibit the lowest error rate of any thermostable DNA Polymerase, a 12-fold increase in fidelity of DNA synthesis compared with *Taq* DNA polymerase. *Psp* DNA polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. *Psp* DNA polymerase generated PCR fragments are blunt-ended, which can be used directly for blunt end ligation. *Psp* DNA Polymerase exhibits lower than that of *Taq* DNA Polymerase extension rate (0.5kb/min), so 2min extension time is recommended for every 1 kb to be amplified.

*Psp* DNA Polymerase prefers MgSO<sub>4</sub> to MgCl<sub>2</sub>.

Error rate (x10<sup>-5</sup>): 0.2

For 50µl volume start from 1.25 – 2.5 units. Meanwhile, for minute amount of template the amount of enzyme should be reduced substantially. For optimal performance use dilutions 1/2, 1/10, 1/50 of the enzyme supplied and use 1 µl of each dilution for the reaction.

One minute extension time is sufficient for PCR fragments up to 0.5-1 kb

**Purity test** *Psp* DNA polymerase is free from endonucleases activities, no traces of bacterial DNA was found in PCR-reactions with primers normally designed for amplification of superconservative region of rDNA

**Storage buffer** 50mM TrisHCl, pH8,2; 0,1 mM EDTA; 1mM DTT; 0,1% Nonidet P40; 0,1% Tween 20; 50% glycerol

**Concentration** 5000 units/ml

#### **Supplied Buffers:**

**INCOMPLETE: (10X)** 200 mM Tris-HCl (pH 8,8), 100 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1,0 % Triton X-100, 1 mg/ml nuclease-free BSA

**COMPLETE: (10X)** 200 mM Tris-HCl (pH 8,8), 100 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM MgSO<sub>4</sub>, 1,0 % Triton X-100, 1 mg/ml nuclease-free BSA

One tube MgSO<sub>4</sub> (100mM)

**Storage conditions** Storage temperature is -20°C.

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**Protocol (example for minute amount of genomic DNA)  
Be careful with the amount of the enzyme!**

*The influence of Psp DNA polymerase amount on the performance of Psp-directed PCR*

PCR was performed in 25µl on Eppendorf Master cycler with the following program:

94°C – 2 min, initial denaturation step

30 cycles:  
94°C – 10sec  
55°C – 20sec  
72°C – 1 min 30sec

72°C – 5 min – final filling-in

Template – Genomic DNA of rodent *Microtus arvalis* (10ng/µl)

Primers:

M7 – 5'-TATGTGCCTTTTCCTATAAGC (20pmol/µl)

T10 – 5'AAGCAGGTATCCATTACC (20pmol/µl)

10pmoles of each primer was used in PCR reaction

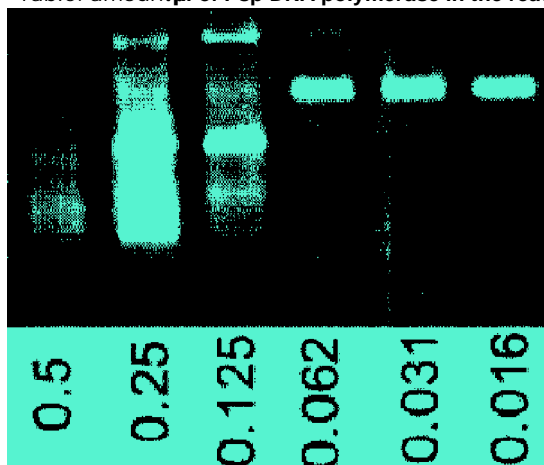
Amplicon – 720bp fragment of Xist gene

10xreaction buffer:  
100mM ammonia sulfate  
200mM TrisHCl, pH 8,8  
100mM KCl  
20mM MgSO<sub>4</sub>  
1% Triton X-100  
1mg/ml BSA

Reaction mixture:

dNTPs mixture (8mM of each) –	0,5 µl
10x buffer	2,5 µl
primer M7 (20 µM)	0,5 µl
primer T10 (20 µM)	0,5 µl
DNA 10ng/µl	1 µl
Psp DNA –polymerase	x µl
Water	up to 25µl

Table: amount µl of Psp DNA polymerase in the reaction.



The optimal amount of Psp DNA polymerase is 0,016-0,062 µl of the enzyme, it corresponds to 0,08-0,3 units of the enzyme per reaction. The increase of enzyme concentrations results in unspecific products formation (0,625-1,3u) and in the complete disappearance of the product at 2,5u.

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