

The ENZYME Company

Pfu DNA polymerase (DNA-tested)

Description *Pfu* DNA polymerase, isolated from the hyperthermophilic archae bacteria *Pyrococcus furiosus* 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 MgSO₄). Unlike *Taq* DNA polymerase, *Pfu* DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that *Pfu* 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. *Pfu* DNA polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. *Pfu* DNA polymerase generated PCR fragments are blunt-ended, which can be used directly for blunt end ligation. *Pfu* 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.

Pfu DNA Polymerase prefers MgSO₄ to MgCl₂.

Error rate (x10⁻⁵): 0.2

For 50µl volume use:

1.25 – 2.5 units working with plasmid DNA (1-2 ng of the template)

0,16 – 0,6 units working with genomic DNA (10 ng of the template)

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

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:

DILUTION BUFFER: TrisHCl, pH8,2; 0,1 mM EDTA; 1mM DTT; 0,1% Nonidet P40; 0,1% Tween 20; 50% glycerol

INCOMPLETE: (10X) 200 mM Tris-HCl (pH 8,8), 100 mM KCl, 100 mM (NH₄)₂SO₄, 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 (NH₄)₂SO₄, 20 mM MgSO₄, 1,0 % Triton X-100, 1 mg/ml nuclease-free BSA

One tube MgSO₄ (100 mM)

Pfu DNA polymerase from Bioron GmbH is proved to be bacterial DNA-free.

Storage conditions Storage temperature is -20°C.

Catalog #	Pack size
108105	500 u
108125	2500 u

BIORON

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Protocol:

Be careful with the amount of the enzyme!

The influence of Pfu DNA polymerase amount on the performance of Pfu-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'-TATGTGCCTTTCCCTATAAGC (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

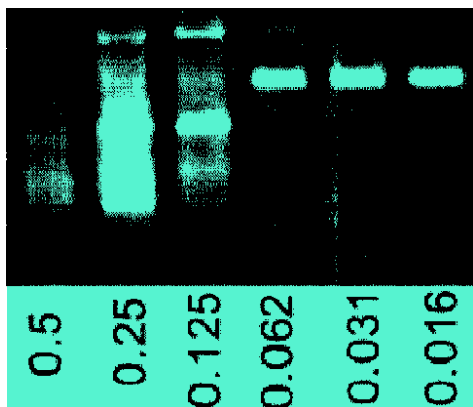
20mM MgSO₄

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
Pfu DNA –polymerase	x µl
Water	up to 25µl



µl of Pfu DNA polymerase in the reaction.

The optimal amount of Pfu 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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