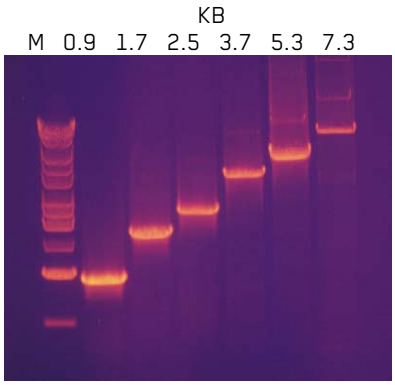
Pfu DNA Polymerase

Pfu DNA Polymerase
(Pyrococcus furiosus)

Cat. No.	Size
E1114-01	100 units
E1114-02	500 units
E1114-03	2500 units

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 min at 74˚C. The reaction conditions are: 50 mM Tris-HCl (pH 9.0 at 25˚C), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^{3}H]dTTP), 10 µg activated calf thymus DNA and 0.1 mg/ml BSA in a final volume of 50 µl.

Storage Conditions:
Store at -20˚C.



PCR amplification using EURx *Pfu* DNA Polymerase. Lane M: molecular weight marker - Perfect 1 kb Ladder. Lanes: 0.9 to 7.3 kb: PCR amplification reaction, using 10x Pfu buffer with 0.25 – 0.3 mM dNTPs and 2.5 U EURx *Pfu* Polymerase in 50 µl (0.2 ml tubes) volume.

Extremely thermostable proofreading DNA polymerase, suitable for applications requiring high temperature synthesis of DNA.

Description:

- *Pfu* DNA Polymerase is a thermostable enzyme isolated from the hyperthermophilic archaebacterium *Pyroccocus furiosus* (1).
- Ultrapure recombinant enzyme.
- Unmodified enzyme replicates DNA at 74°C and exhibits over 95% activity after 1-hour incubation at 95˚C.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium ions.
- The enzyme also exhibits the 3' → 5'' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with *Taq* DNA Polymerases (2).
- *Pfu* DNA Polymerase is recommended for use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures, primer extension reactions at elevated temperatures and cloning of blunt-ended PCR products.

Storage Buffer:

50 mM Tris-HCl (pH 8.2 at 22˚C), 0.1 Tween 20, 0.1% Igepal, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

10 x Reaction Buffer:

10 x Pfu Buffer
The buffer contains 20 mM $MgSO_4$.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. *Lundberg, K., Shoemaker, D., Adams, M., Short, J., Sorge, J. and Mathur E. (1991) Gene 108, 1.*
2. *Cline, J., Braham, J. and Hogrefe, H. (1996) Nucleic Acids Res. 24, 3546.*

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | 13125 BERLIN | GERMANY
FAX +4930-31019197 | PHONE +4930-31809372 | INTERNETSHOP WWW.ROBOKLON.DE
MANUFACTURED BY EURx Sp. z o.o. POLAND | MADE IN THE EUROPEAN UNION

Pfu DNA Polymerase

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
10 x *Pfu* Buffer, containing 20 mM $MgSO_4$.	5 µl	1x
dNTP mix (5mM each)	2.0 – 3.0 µl	0.2-0.3 mM each dNTP
Upstream primer	Variable	0.2-0.5 µM
Downstream primer	Variable	0.2-0.5 µM
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Pfu DNA Polymerase, 5 U/µl	0.5 µl	2.5 U
Total volume	50 µl	-

Thermal Cycling Conditions:

Step	Tempe-rature	Time	Number of Cycles
Initial Denaturation	94-95˚C	2-5 min	1
Denaturation	94-95˚C	15 - 60 s	25-35
Annealing	50-68˚C	30 - 60 s	
Extension	72˚C	**1 min/1 kb**	
Final Extension	72˚C	7 min	1
Cooling	4˚C	Indefinite	1

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. This is especially important for magnesium solutions, because they form a concentration gradient when frozen.
2. Prepare reaction mixes on ice.
3. Place reactions in a thermal cycler that has been preheated to 94-95˚C.
4. *Pfu* DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading activity of Pfu DNA Polymerase may degrade primers.
5. For *Pfu* DNA Polymerase-based PCR, the standard concentration of $MgSO_4$ is 2 mM (as provided by the 1 x *Pfu* Buffer). In most cases this concentration will produce satisfactory results. Should the reaction require inceased Mg^{2+} concentrations, use the supplied 25 mM $MgSO_4$ solution for adjustment.
6. Use 0.2-0.3 mM dNTP (each) per PCR reaction. (see Table: Preparation of PCR Reaction). The optimal dNTP concentration may vary between individual reactions. If you are not satisfied with the final yield, the dNTPs concentration should be adjusted up to 0.3 mM (try 0.2, 0.25, 0.3 mM). In many cases increasing the concentration to 0.3 mM allows to achieve the best yield while preserving specificity and fidelity of Pfu DNA Polymerase.
7. 2.5 U of *Pfu* Plus! DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
8. The amount of DNA template required depends on the type of DNA being amplified. Generally, 50-250 ng of genomic DNA, 1-50 ng of plasmid or phage DNA or 10-100 ng of multicopy chromosomal genes are recommended.

Notes:

1. Annealing temperature should be optimized for each primer set based on the primer T_m. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5˚C below T_m.
2. The recommended extension time is 1 min/1 kb to be amplified.

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