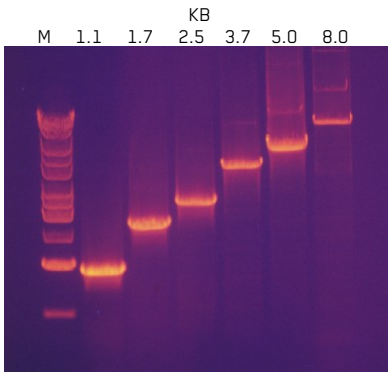
Blue *Pfu* DNA Polymerase

Pfu DNA Polymerase
(Pyrococcus furiosus)

Cat. No.	Size
E1116-01	100 units
E1116-02	500 units
E1116-03	2 500 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 or at -70 °C.



PCR amplification using EURx Blue *Pfu* DNA Polymerase. Lane M: molecular size marker - Perfect 1 kb Ladder. Lanes: 1.1 to 3.7 kb: PCR amplification reaction, using buffer B with 0.1 mM dNTPs and 2.5 u EURx *Pfu* Polymerase in 50 µl (0.2 ml tubes) volume, 1 ng lambda DNA as a template, under cycling regime: 94°C 2 min, followed by 35 cycles of 94°C for 20 sec, 55°C for 30 sec and 72°C 1 min/1 kb. Lane 5 kb: PCR as above, using 0.2 mM dNTPs and 3% DMSO. Lane 8 kb: buffer C with 0.35 mM dNTPs, 3% DMSO and 3.75 u EURx *Pfu* Polymerase.

Extremely thermostable proofreading DNA polymerase, suitable for applications requiring high temperature synthesis of DNA. The enzyme is supplemented with an inert blue tracer dye.

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 98°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.
- Added blue tracer dye offers several advantages:
  - + visualizes the addition of the polymerase to the reaction;
  - + confirms complete mixing;
  - + allows for direct loading onto the gel, without the need to add loading buffers and thus saving one step in multiple PCR reactions;
  - + serves as tracking dye during gel electrophoresis;
  - + has no effect on subsequent DNA manipulations such as: ligation, transformation, automated DNA sequencing, restriction digestion;
- overcomes a frequent problem associated with the red dye, used by other manufacturers, which strongly quenches fluorescence of those DNA bands on the gel, where migration overlaps with the red dye.

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 AmpliBuffer A (optimization buffer without $MgCl_2$):
500 mM KCl, 100 mM Tris-HCl (pH 9.1 at 20°C), 0.1% Triton X-100 and stabilizers. The buffer is optimized for use with 0.1-0.2 mM of each dNTP.

10 x AmpliBuffer B (general application, up to 6-8 kb):
500 mM KCl, 100 mM Tris-HCl (pH 9.1 at 20°C), 0.1% Triton X-100, 15 mM $MgCl_2$ and stabilizers. The buffer is optimized for use with 0.1-0.2 mM of each dNTP.

10 x AmpliBuffer C (for products over 6-8 kb):
160 mM $(NH_4)_2SO_4$, 500 mM Tris-HCl (pH 9.2 at 22˚C), 17.5 mM $MgCl_2$ and stabilizers. The buffer is optimized for use with 0.35 mM of each dNTP.

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.*

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