EURx® MOLECULAR BIOLOGY PRODUCTS

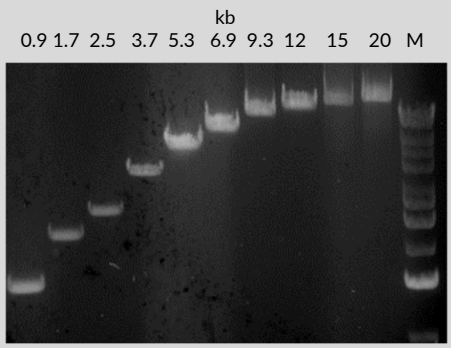
Color PfuPlus!
DNA Polymerase

(Pyrococcus sp.)

Cat. No.	size
E1110-01	100 units
E1110-02	500 units
E1110-03	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 minutes 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 Color PfuPlus! DNA Polymerase. Lanes 0.9-5.3 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.2 mM dNTPs and 2.5 U EURx Color PfuPlus! DNA Polymerase in 50 µl reaction volume. Lanes 6.9-20 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.25 mM dNTPs and 2.5 U EURx Color PfuPlus! DNA Polymerase in 50 µl reaction volume. Lane M: molecular size marker - Perfect 1 kb DNA Ladder.

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.*
3. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) Biokhimiya 45, 644. (1980) Biokhimiya 45, 644.*

Extremely thermostable proofreading DNA polymerase blend, formulated for efficient site-directed mutagenesis and synthesis of DNA products up to 20 kb.

The enzyme is supplemented with two inert gel tracking dyes.

Description:

- PfuPlus! is a modified and optimized hyperthermophilic Pfu DNA Polymerase (1) blended with the thermostable polymerase-enhancing factor.
- Ultrapure recombinant enzyme.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases (2).
- The constituent of PfuPlus! DNA Polymerase, the polymerase-enhancing factor, enhances PCR product yields and increases target length capability of Pfu DNA Polymerase.
- Enhanced performance of PfuPlus! DNA Polymerase allows to use fewer PCR cycles and lower DNA template concentrations when compared to Pfu DNA Polymerase.
- Color PfuPlus! DNA Polymerase is recommended for use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures, site-directed mutagenesis and cloning of blunt-ended PCR products.
- The enzyme is also recommended for general use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of PCR products up to 20 kb.
- Use of Color PfuPlus! DNA Polymerase offers several advantages:
    - visualizes the addition of the polymerase to the reaction,
    - confirms complete mixing,
    - enables direct loading of PCR products onto an agarose gel without addition of a gel loading buffer,
    - the added dyes allow to track electrophoresis progress,
    - do not affect PCR performance,
    - do not interfere with most downstream applications (exception: the polymerase is not recommended for any downstream applications using absorbance or fluorescence excitation).

Storage Buffer:

50 mM Tris-HCl (pH 8.0 at 22°C), 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

10 x Pfu Buffer:

The buffer contains 15 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.

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Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Pfu Buffer contains 15 mM $MgSO_4$	5 µl	1x
dNTP mix (5 mM each)	2.0-2.5 µl	0.2-0.25 mM of each dNTP
Upstream primer	Variable	0.2-0.5 µM
Downstream primer	Variable	0.2-0.5 µM
Color PfuPlus! DNA Polymerase, 1.25 U/µl	2 µl	2.5 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled	Variable	-
Total volume	50 µl	-

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradient when frozen.
2. Prepare reaction mixes on ice. Mix well.
3. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. Color PfuPlus! DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading activity of Color PfuPlus! DNA Polymerase may degrade primers.
5. For Color PfuPlus! DNA Polymerase-based PCR, standard concentration of $MgSO_4$ is 1.5 mM (as provided by the 1 x Pfu Buffer). In most cases this concentration will produce satisfactory results. If increased Mg^{2+} concentration is needed use 25 mM $MgSO_4$ provided.
6. Use of Color PfuPlus! DNA Polymerase allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The polymerase contains two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
7. The optimal concentration of dNTPs in PCR reactions should be adjusted empirically and depends on the amplicon length. Longer targets usually amplify better with 0.25 mM dNTPs.
8. 2.5 U of Color PfuPlus! DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. For some PCR targets further optimization will be required. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
9. A minimum of 1.5 µl of Color PfuPlus! DNA Polymerase must be added per 50 µl reaction to enable direct loading of PCR products onto an agarose gel without addition of a gel loading buffer.
10. In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO can be included to improve amplification. Use DMSO in concentrations of 2-8%. The recommended starting DMSO concentration (if needed) is 3%.
11. The amount of DNA template required depends on the type of DNA being amplified. Generally 50-250 ng of genomic DNA, 0.1-10 ng of plasmid DNA, 1-20 ng of phage DNA and 10-100 ng of multicopy chromosomal genes is recommended.

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Thermal Cycling Conditions for PCR Products up to 6 kb

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation	94-95°C	20-30 s	25-35
Annealing	50-68°C	30 s	
Extension	72°C	**1 min/1 kb**	
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Thermal Cycling Conditions for PCR Products over 6 kb

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94°C	2 min	1
Denaturation	94°C	10-15 s	10
Annealing	60-68°C	30 s	
Extension	68°C	1 min/1 kb	
Denaturation	94°C	10-15 s	20-25
Annealing	60-68°C	30 s	
Extension	68°C	**1 min/1 kb +20 s in each additio-**	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

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. Typical primers for long PCR amplification have a length of 22-34 and should have annealing temperatures above 60°C to enhance reaction specificity.
3. When amplifying long PCR products keep denaturation steps as short as possible and denaturation temperature as low as possible (try not to exceed 2 min at 94°C during initial denaturation and 10-15 s at 94°C during cycle denaturation step). Sometimes fragments with high GC-content need higher denaturation temperatures, but keep in mind that the yield increases when denaturation temperature/duration is decreased.
4. For PCR products over 6 kb use elongation temperature of 68°C instead of 72°C.
5. For PCR products over 6 kb elongation of extension step (+20 s in each additional cycle starting from 11th cycle) is recommended due to loss of processivity of the enzyme.

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Site-directed mutagenesis

Preparation of Mutagenesis Reaction:

Component	Volume/reaction	Final concentration
10 x Pfu Buffer contains 15 mM $MgSO_4$	5 µl	1x
dNTP mix (5 mM each)	2.0-2.5 µl	0.2-0.25 mM of each
Mutagenic primer #1	Variable	0.2 µM
Mutagenic primer #2	Variable	0.2 µM
Color PfuPlus! DNA Polymerase, 1.25 U/µl	2 µl	2.5 U
Plasmid DNA template	Variable	5-50 ng
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Thermal Cycling Conditions for Site-Directed Mutagenesis

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	1 min	1
Denaturation	95°C	30 s	18
Annealing	X°C	30-60 s	
Extension	68°C	**1 min/1 kb**	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. Completely thaw and mix thoroughly all components of mutagenesis reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradient when frozen.
2. Prepare reaction mixes on ice. Mix well.
3. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. Color PfuPlus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading activity of Color PfuPlus! DNA Polymerase may degrade primers.
5. The recommended concentration of dNTPs used in site-directed mutagenesis is 0.2-0.25 mM dNTPs irrespective of plasmid length.
6. 2.5 U of Color PfuPlus! DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For some mutagenesis targets further optimization will be required.
7. The mutagenesis protocol usually requires 5-50 ng of plasmid DNA to achieve satisfactory results.
8. Both of the mutagenic primers must contain the intended mutation and anneal to the same sequence on opposite strands of the plasmid. The intended mutation should be in the middle of primer with at least 10 bases of correct sequence on both sides.
9. The mutagenic primers should be used in concentration of 0.2 µM each per reaction.

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