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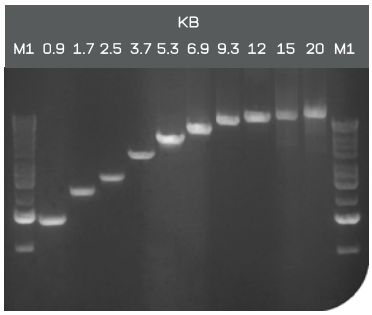
Color *Pfu* Plus! DNA Polymerase

Pfu DNA Polymerase
(Pyrococcus furiosus)
MODIFIED

Cat. No.	Size
E1110-01	100 units
E1110-02	500 units
E1110-03	2 500 units

Storage Conditions:
Store at -20°C.



PCR amplification using EURx Color *Pfu*Plus! DNA Polymerase.
Lanes 0.9 to 5.3 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.2 mM dNTPs and 2.5 U EURx Color *Pfu*Plus! DNA Polymerase in 50 µl reaction volume.
Lanes 6.9-12.1 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.3 mM dNTPs and 2.5 U EURx Color *Pfu*Plus! DNA Polymerase in 50 µl reaction volume.
Lanes 15-20 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.35 mM dNTPs and 2.5 U EURx Color *Pfu*Plus! DNA Polymerase in 50 µl reaction volume.
Lane M1: molecular size marker - Perfect 1 kb DNA Ladder (E3130).

Extremely thermostable proofreading DNA polymerase blend, formulated for efficient site-directed mutagenesis and synthesis of ultra wide range of DNA products up to 20 kb in length. The enzyme is supplemented with two inert gel tracking dyes.

Description:

- Color *Pfu*Plus! is a modified and optimized hyperthermostable *Pfu* DNA Polymerase (1) blended with thermostable polymerisation enhancing factors.
- Ultrapure recombinant enzymes mixture.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium ions.
- The enzyme exhibits 3´→5´ proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with *Taq* DNA Polymerases (2).
- A constituent of Color *Pfu*Plus! DNA Polymerase, the polymerase-enhancing factor, enhances PCR product yields and increases target length capability of Pfu DNA Polymerase.
- The enhanced performance of Color *Pfu*Plus! DNA Polymerase allows to use fewer PCR cycles and lower DNA template concentrations, as compared to Pfu DNA Polymerase.
- Color *Pfu*Plus! is recommended for use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures, primer extension reactions at elevated temperatures, site-directed mutagenesis and cloning of blunt-ended PCR products.
- Color *Pfu*Plus! DNA Polymerase is also recommended for general use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products from several hundred bp to over 20 kb.
- Use of Color *Pfu*Plus! 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,
  - serves as tracking dye during gel electrophoresis;
  - the added dyes allow to track electrophoresis progress,
  - the added dyes do not affect PCR performance,
  - do not interfere with most downstream applications (exception: is not recommended for any downstream applications using absorbance or fluorescence excitation).

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 Buffer:

50 mM Tris-HCl (pH 8.2 at 22°C), 0.1 mM EDTA, 1 mM dithiothreitol, 50 % (v/v) glycerol and stabilizers.

10 x Reaction Buffer:

10 x Pfu Buffer
The buffer contains 15 mM $MgSO_4$.

Quality Control:

All preparations are assayed for contaminating 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.*
3. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) Biokhimiya 45, 644.*

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Color *Pfu* Plus! DNA Polymerase

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x *Pfu* Buffer, containing 15 mM $MgSO_4$.	5 µl	1x
dNTP mix (5mM each)	2.0 – 2.5 µl	0.2-0.25 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	-
Color *Pfu* Plus! DNA Polymerase, 1 U/µl	2.5 µl	2.5 U
Total volume	50 µl	-

Thermal Cycling Conditions for Products up to 6 kb in Size:

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 Products Larger Than 6 kb in Size:

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	25-35
Annealing	60-68˚C	30 s	
Extension	68˚C	**1 min/1 kb +20 s per additional cycle**	
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Notes:

1. **Concentration Differences:** 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. **On Ice**: Prepare reaction mixes on ice. Mix well.
3. **Preheat Cycler:** Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. **Add Enzyme as Last Component:** Color *Pfu* Plus! DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading (exonuclease) activity of PfuPlus! DNA Polymerase may degrade primers.
5. **$MgSO_4$:** For Color *Pfu* Plus! 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.
   Adding 1 µl of a 25 mM $MgSO_4$ solution to a total reaction volume of 50 µl will add 25 nmol $MgSO_4$ and thus increase total $MgSO_4$ reaction concentration in 0.5 mM.
   Increasing the $MgSO_4$ concentration enhances PCR yield but decreases reaction specificity (amplification of more bands, but also of non-specific bands). Decreasing the $MgSO_4$ concentration decreases PCR yield but enhances reaction specificity (less bands, but specific PCR products).
6. **Colored enzyme mix:** Use of Color *Pfu*Plus! 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 an 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. **dNTP Concentration:** The recommended concentration of dNTPs used in PCR reactions depends on the amplicon length and should be adjusted empirically. Good results for long targets are usually achieved by using a dNTP concentration of 0.25 mM.
8. **Amount of Enzyme:** 2.5 U of Color *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. In some cases it may be necessary to optimize the enzyme concentration. Excess amounts of enzyme may generate artifacts like as smearing of bands, etc.
9. **Minimum Amount of Enzyme:** A minimum of 1.5 µl of Color *Pfu*Plus! 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. **Template DNA Amount:** 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 or 10-100 ng of multicopy chromosomal genes is recommended.

Notes:

1. **Annealing:** 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. **Long PCR – Primer Requirements:** Typical primers for long PCR amplification reactions have a length of 22-34 bp and should have annealing temperatures above 60˚C to enhance reaction specificity.
3. **Long PCR – Short Denaturation Steps:** 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. **Long PCR – Low Elongation Temperature:** For PCR products exceeding 6 kb in length use an elongation temperature of 68˚C rather than 72˚C.
5. **Long PCR – Extended Elongation Period:** For PCR products exceeding 6 kb in length, an elongation of the extension step (+20 s in each additional cycle, starting from the 11th cycle) is strongly recommended due to loss of processivity of the enzymes blend.

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Color *Pfu* Plus! DNA Polymerase
SITE DIRECTED MUTAGENESIS PROTOCOL

Preparation of Mutagenesis Reaction:

Component	Volume/reaction	Final concentration
10 x *Pfu* Buffer, containing 20 mM $MgSO_4$.	5 µl	1x
dNTP mix (5mM each)	2.0-2.5 µl	0.2-0.25 mM of each dNTP
Mutagenic primer #1, 100 ng / ul	Variable	0.2 µM
Mutagenic primer #2, 100 ng / ul	Variable	0.2 µM
Plasmid DNA Template	Variable	5-50 ng
Sterile double-distilled water	Variable	-
Color *Pfu* Plus! DNA Polymerase, 1 U/µl	2.5 µl	2.5 U
Total volume	50 µl	-

Notes:

1. **Concentration Differences:** 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 gradients, when frozen.
2. **On Ice**: Prepare reaction mixes on ice.
3. **Preheat Cycler:** Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. **Add Enzyme as Last Component:** Color *Pfu* Plus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading (= exonuclease) activity of PfuPlus! DNA Polymerase may degrade primers.
5. **dNTP Concentration:** The recommended concentration of dNTPs used in site-directed mutagenesis is 0.2-0.25 mM dNTPs (each), irrespective of plasmid length.
6. **Amount of Enzyme:** 2.5 U of Color *Pfu* Plus! DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For some mutagenesis targets, further optimization will be required.
7. **Target DNA Amount:** The mutagenesis protocol usually requires 5-50 ng of plasmid DNA to achieve satisfactory results.
8. **Placement of Intended Mutation:** 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. **Amount of Mutagenic Primers:** The mutagenic primers should be used in a concentration of 0.2 µM each per reaction.

Thermal Cycling Conditions:

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

Notes:

1. **Annealing Temperature:** Adjust the annealing temperature accordingly. As a guideline for orientation: Often, the annealing temperature ranges between 55-60°C, but may differ from these values for certain templates. As a good starting point, use 55°C.

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