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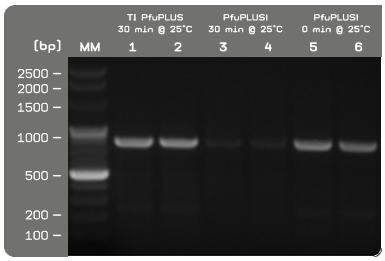
TI *Pfu* Plus! DNA Polymerase

Pfu DNA Polymerase
(Pyrococcus furiosus)
MODIFIED

Cat. No.	Size
E1113-01	100 units
E1113-02	500 units
E1113-03	2 500 units

Storage Conditions:
Store at -20°C.



PCR amplification using EURx ti*Pfu*Plus! DNA Polymerase - demonstration of "HotStart" capabilities.
A 0.82 kb amplicon of the human beta-globin gene was amplified using EURx ti*Pfu*Plus! DNA Polymerase, 10 x Pfu Buffer and 0.2 mM dNTPs in 50 µl reaction volume. To demonstrate the impact of "Hot Start" on PCR-yield and PCR-specificity, both, "Hot Start" (lanes 1, 2) and non-"Hot Start" assays (lanes 3, 4) were incubated at 25°C for 30 min before performing PCR cycling.
Lane MM: Molecular size marker – Perfect 100 bp DNA Ladder (Cat. no. E3134).
Lanes 1,2: PCR amplification reactions using 2.5 U ti*Pfu*Plus! DNA Polymerase(TI-inhibitor mediated "Hot Start". Reactions were incubated 30 min at 25°C before PCR.
Lanes 3, 4: PCR amplification reactions using 2.5 U non-"Hot Start" *Pfu*Plus! DNA Polymerase. Reactions were incubated 30 min at 25°C before PCR.
Lanes 5, 6: PCR amplification reactions using 2.5 U non-"Hot Start" *Pfu*Plus! DNA Polymerase. Reactions were set up on ice.

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.

Description:

- ti*Pfu*Plus! DNA polymerase is a new generation "HotStart" enzyme blend. Enzyme activity is blocked at moderate temperatures and allows room temperature reaction setup. DNA polymerase actvity is restored during normal PCR cycling conditions.
- Use of ti*Pfu*Plus! DNA Polymerase allows for an enormous increase of PCR specificity, sensitivity and yield, as compared to conventional PCR assembly methods.
- ti*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 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 ti*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 ti*Pfu*Plus! DNA Polymerase allows to use fewer PCR cycles and lower DNA template concentrations, as compared to Pfu DNA Polymerase.
- ti*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.
- ti*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.

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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TI *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	-
ti*Pfu*Plus! DNA Polymerase, 5 U/µl	0.5 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. **Room Temperature.** Setup reactions at room temperature. Use of ti*Pfu*Plus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. **Cycler Preheating Not Required.** Reactions can be placed in a room temperature thermal cycler.
4. **Add Enzyme as Last Component:** ti*Pfu*Plus! DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading (exonuclease) activity of ti*Pfu*Plus! DNA Polymerase may degrade primers.
5. **$MgSO_4$:** For ti*Pfu*Plus! DNA Polymerase-based PCR, the 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. 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. **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 (rather than 0.2 mM).
7. **Amount of Enzyme:** 2.5 U of ti*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.
8. **Additives / PCR Enhancers.** In most cases there is no need to add additives to the PCR reaction. For some difficult targets such as GC-rich sequences and targets with complex secondary structures, additives such as DMSO may be included to improve amplification. Use DMSO in a concentration range between 2-8% [v/v]. The recommended starting DMSO concentration (if required) is 3% [v/v].
9. **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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SITE DIRECTED MUTAGENESIS PROTOCOL

Preparation of Mutagenesis Reaction:

Component	Volume/reaction	Final concentration
10 x *Pfu* Buffer, containing 1.5 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	Variable	0.2 µM
Mutagenic primer #2	Variable	0.2 µM
Plasmid DNA Template	Variable	5-50 ng
Sterile double-distilled water	Variable	-
ti*Pfu*Plus! DNA Polymerase, 5 U/µl	0.5 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. **Room Temperature.** Setup reactions at room temperature. Use of ti*Pfu*Plus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. **Cycler Preheating Not Required.** Reactions can be placed in a room temperature thermal cycler.
4. **Add Enzyme as Last Component:** ti*Pfu*Plus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading (= exonuclease) activity of ti*Pfu*Plus! DNA Polymerase may degrade primers.
5. **Amount of Enzyme:** 2.5 U of ti*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.
6. **Target DNA Amount:** The mutagenesis protocol usually requires 5-50 ng of plasmid DNA to achieve satisfactory results.
7. **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 primers, respectively, with at least 10 bases of correct sequence on both sides.
8. **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	30 s-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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