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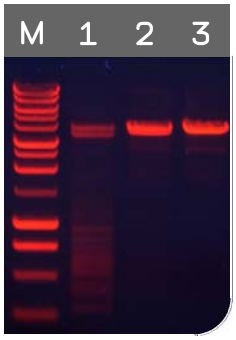
on*Pfu* Plus! DNA Polymerase

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 on*Pfu*Plus! DNA Polymerase.
A 4 kb amplicon of the human beta-globin gene was amplified using EURx on*Pfu*Plus! DNA Polymerase, 10 x on*Pfu* Buffer and 0.2 mM dNTPs in a 50 µl reaction volume.
Lane M: molecular size marker – Perfect 1 kb DNA Ladder.
Lane 1: PCR amplification reaction using 2.5 U *Pfu*Plus! DNA Polymerase (E1118). To demonstrate "Hot Start", the reaction was incubated 30 min at 25˚C before PCR – against the recommendations given in the protocol.
Lane 2: PCR amplification reaction using 2.5 U PfuPlus! DNA Polymerase (E1118). Reaction was set up on ice, in accordance with the supplied protocol.
Lane 3: PCR amplification reaction using 2.5 U on*Pfu*Plus! DNA Polymerase. To demonstrate "Hot Start", the reaction was incubated 30 min at 25˚C before PCR.

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:

- on*Pfu*Plus! is a modified and optimized hyperthermostable *Pfu* DNA Polymerase (1) blended with thermostable polymerisation enhancing factors.
- Hot Start: Enzyne activity is blocked at moderate temperatures. Polymerase activity is restored irreversibly during the initial denaturation step, when amplificatin reactions are heated to 95˚C for 10 minutes.
- Automated "Hot Start" increases PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method
- 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 on*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 on*Pfu*Plus! DNA Polymerase allows to use fewer PCR cycles and lower DNA template concentrations, as compared to Pfu DNA Polymerase.
- *on*PfuPlus! 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.
- on*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 8.0 at 22˚C), 0.1 mM EDTA, 1 mM dithiothreitol, 50 % (v/v) glycerol and stabilizers.

10 x Reaction Buffer:

10 x onPfu 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.

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PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x on*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	-
on*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	95˚C	10 min	1
Denaturation	94˚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	95˚C	10 min	1
Denaturation	94˚C	15 s	10
Annealing	60-68˚C	30 s	
Extension	68˚C	1 min/1 kb	
Denaturation	94˚C	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 Trempereature Assembly**: Prepare reaction mixes at room temperature. Use of onPfuPlus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler.
4. **Add Enzyme as Last Component:** on*Pfu*Plus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading (= exonuclease) activity of on*Pfu*Plus! DNA Polymerase may degrade primers.
5. **$MgSO_4$:** For on*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.
7. **Amount of Enzyme:** 2.5 U of on*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, sequences with complex secondary structures and long targets additives such as DMSO (provided) can be included to improve amplification. The recommended starting DMSO concentration (if needed) 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. **Initial Denaturation:** on*Pfu*Plus! DNA Polymerase is activated by a 10-minute initial denaturation step at 95˚C.
2. **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.
3. **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.
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 on*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	-
on*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 Trempeature Assembly**: Prepare reaction mixes at room temperature. Use of onPfuPlus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler.
4. **Add Enzyme as Last Component:** on*Pfu*Plus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading (= exonuclease) activity of on*Pfu*Plus! 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 on*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	10 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. **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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