EURx® MOLECULAR BIOLOGY PRODUCTS

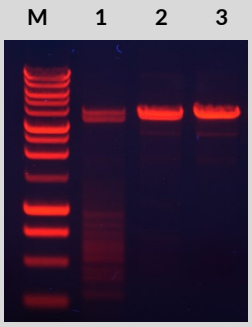
onPfuPlus! DNA Polymerase HOT START

(Pyrococcus sp.)

Cat. No.	size
E1113-01	100 units
E1113-02	500 units
E1113-03	2500 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 onPfuPlus! DNA Polymerase. A 4 kb amplicon of the human β-globin gene was amplified using EURx onPfuPlus! DNA Polymerase, 10 x onPfu Buffer and 0.2 mM dNTPs in 50 µl reaction volume.

Lane M: molecular size marker – Perfect 1 kb DNA Ladder.

Lane 1: PCR amplification reaction using 2.5 U PfuPlus! DNA Polymerase. Reaction was incubated 30 min at 25°C before PCR.

Lane 2: PCR amplification reaction using 2.5 U PfuPlus! DNA Polymerase. Reaction was set up on ice.

Lane 3: PCR amplification reaction using 2.5 U onPfuPlus! DNA Polymerase. Reaction was incubated 30 min at 25°C before PCR.

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

Description:

- onPfuPlus! DNA Polymerase is a modified hot start enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 10 minutes.
- Use of onPfuPlus! DNA Polymerase allows for the increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- onPfuPlus! is a modified and optimized hyperthermophilic Pfu DNA Polymerase 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.
- The constituent of onPfuPlus! DNA Polymerase, the polymerase-enhancing factor, enhances PCR product yields and increases target length capability of Pfu DNA Polymerase.
- onPfuPlus! 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.

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 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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Preparation of PCR reaction:

Component	Volume/reaction	Final concentration
10 x onPfu 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
onPfuPlus! DNA Polymerase, 5 U/µl	0.5 µl	2.5 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	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 at room temperature. Use of onPfuPlus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. For onPfuPlus! DNA Polymerase-based PCR, standard concentration of $MgSO_4$ is 1.5 mM (as provided by the 1 x onPfu Buffer). In most cases this concentration will produce satisfactory results. If increased Mg^{2+} concentration is needed use 25 mM $MgSO_4$ provided.
5. 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.
6. 2.5 U of onPfuPlus! 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.
7. 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%.
8. 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	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 PCR Products over 6 kb

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 in each additio-**	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. onPfuPlus! DNA Polymerase is activated by a 10-minute initial denaturation step at 95°C.
2. 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. Typical primers for long PCR amplification have a length of 22 -34 and should have annealing temperatures above 60°C to enhance reaction specificity.
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 onPfu 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
Mutagenic primer #1	Variable	0.2 µM
Mutagenic primer #2	Variable	0.2 µM
onPfuPlus! DNA Polymerase, 5 U/µl	0.5 µl	2.5 U
Plasimd DNA template	Variable	5-50 ng
Sterile double-distilled	Variable	-
Total volume	50 µl	-

Thermal Cycling Conditions for Site-Directed Mutagenesis

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. 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 at room temperature. Use of onPfuPlus! DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. onPfuPlus! DNA Polymerase should be the last component added to the mutagenesis mixture. In the absence of dNTPs proofreading activity of onPfuPlus! DNA Polymerase may degrade primers.
5. 2.5 U of onPfuPlus! DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For some mutagenesis targets further optimization will be required.
6. The mutagenesis protocol usually requires 5-50 ng of plasmid DNA to achieve satisfactory results.
7. 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.
8. The mutagenic primers should be used in concentration of 0.2 µM each per reaction.

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