EURx® MOLECULAR BIOLOGY PRODUCTS

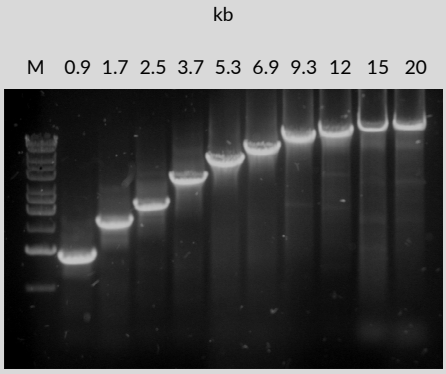
Perpetual OptiTaq DNA Polymerase HOT START

Monoclonal antibody automatic "hot start"

Cat. No.	size
E2720-01	200 units
E2720-04	500 units
E2720-02	1000 units
E2720-03	5000 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 Perpetual OptiTaq DNA Polymerase.

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

Lanes 0.9 to 20 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U Perpetual OptiTaq DNA Polymerase in 50 µl reaction volume.

References:

1. *Cline, J., Braham, J. and Hogrefe, H. (1996) Nucleic Acids Res. 24, 3546.*
2. *Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550.*
3. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) Biokhimiya 45, 644.*

Mixture of thermostable Taq DNA Polymerase, proofreading *Pyrococcus sp.* DNA Polymerase, anti-Taq DNA Polymerase antibodies for automatic "hot start" PCR. The blend generates products up to 20 kb with stringent amplification specificity, sensitivity, fidelity and yield.

Description:

- Perpetual OptiTaq DNA Polymerase is a modified and balanced blend containing top quality *Thermus aquaticus* DNA Polymerase, *Pyrococcus sp.* DNA Polymerase and anti-Taq DNA Polymerase antibodies.
- Ultrapure, recombinant proteins are used to prepare Perpetual OptiTaq DNA Polymerase.
- Our carefully selected anti-Taq antibodies have high thermal stability, providing protection against non-specific primer extension from room temperature to 70°C.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 92-95°C for two minutes.
- Formation of inactive complexes between Taq DNA Polymerase and an anti-Taq antibody forms a basis for automatic "hot start" PCR, which allows for the assembly of PCR reactions at room temperature.
- High stability of the complexes allows for the enormous increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- Automatic "hot start" PCR is a convenient method when assembling multiple PCR reactions, saving time and efforts.
- Clean and safe laboratory practice assured, due to removed necessity to open hot tubes.
- The blend catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions and exhibits the 3'→5' proofreading activity, resulting in considerably higher PCR fidelity and processivity than possible with unmodified Taq DNA polymerase (1).
- Enables increased amplification product yield in comparison with Taq DNA polymerase over wide range of PCR products.
- Maintains the 5'→3' exonuclease activity.
- Adds extra A at the 3' ends.
- Suitable for multiplex PCR due to increased specificity, wider tolerance for Mg^{2+}, salts concentration and pH (2,3).
- Improves PCR results with critical templates, such as containing GC-rich regions, palindromes or multiple repeats.
- Perpetual OptiTaq DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products up to 20 kb.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

10 x Reaction Buffers:

10 x Pol Buffer A (optimization buffer without $MgCl_2$):

The buffer allows to optimize $MgCl_2$ concentration.

10 x Pol Buffer B (general application, up to 20 kb):

The buffer contains 15 mM $MgCl_2$ and is optimized for use with 0.2 mM of each dNTP.

10 x Pol Buffer C (colored):

10 x Pol Buffer B enriched with two gel tracking dyes and a gel loading reagent. The buffer enables direct loading of PCR products onto an agarose gel.

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 Pol Buffer A or 10 x Pol Buffer B or 10 x Pol Buffer C	5 µl	1x
25 mM $MgCl_2$	2-10 µl when using 10 x Pol Buffer A 0-7 µl when using 10 x Pol Buffer B or 10 x Pol Buffer C	1-5 mM 1.5-5 mM
dNTP mix (5 mM each)	2 µl	0.2 mM of each dNTP
Upstream primer	Variable	0.3-0.5 µM
Downstream primer	Variable	0.3-0.5 µM
Perpetual OptiTaq DNA Polymerase, 2.5 U/µl	0.5 µl	1.25 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 Perpetual OptiTaq DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided by the 1 x Pol Buffer B or the 1 x Pol Buffer C) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, in some cases, reaction may be improved by determining optimal concentration of $MgCl_2$.
5. The 10 x Pol Buffer C allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The buffer contains a gel loading reagent and 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.
6. 1.25 U of Perpetual OptiTaq 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. In some cases it may be necessary to optimize the enzyme concentration.
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. As a general guide for how much template DNA to use, start with a minimum 10^4 copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.1 x 10^{11} molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10^8 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules).
9. For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
10. Ensure that a template DNA is of sufficiently high quality. Use only high-molecular-weight DNA, when amplifying long PCR targets (over 20-50 kb, depending on the amplicon length).
11. Complex genomic DNA should be stored at 2-8°C. Avoid vortexing the genomic DNA.
12. Use only thin-walled 0.2 ml tubes performing long PCR amplification.

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Thermal Cycling Conditions for Products 0.1-10 kb

Step	Temperature	Time	Number of Cycles
Initial Denaturation	93-95°C	2-5 min	1
Denaturation	93-95°C	15-30 s	25-35
Annealing	50-68°C	30 s	
Extension	72°C or 68°C	1 min/1 kb	
Final Extension	72°C or 68°C	7 min	1
Cooling	4°C	Indefinite	1

Thermal Cycling Conditions for Products over 10 kb

Step	Temperature	Time	Number of Cycles
Initial Denaturation	92-94°C	2 min	1
Denaturation	92-94°C	10-15 s	10
Annealing	60-68°C	30 s	
Extension	68°C	1 min/1 kb	
Denaturation	92-94°C	10-15 s	15-25
Annealing	60-68°C	30 s	
Extension	68°C	1 min/1 kb +20 s in each additional cycle	
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. 2 min initial denaturation step at 92-95°C is required to inactivate the antibody and restore the polymerase activity.
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. 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.
5. For PCR products over 5 kb elongation temperature of 68°C is strongly recommended.
6. For PCR products over 10 kb elongation of extension step (+20 s in each additional cycle starting from 11th cycle) is strongly recommended due to loss of activity of the enzymes blend.

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