Perpetual Opti*Taq* DNA Polymerase

Monoclonal antibody automatic "Hot Start" PCR system

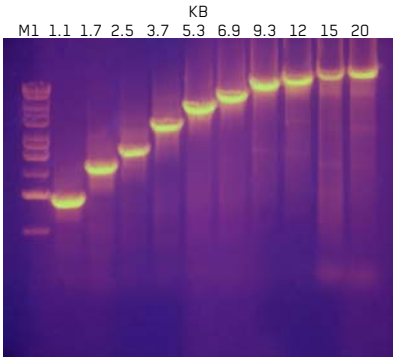
Taq DNA Polymerase
(Thermus aquaticus)
Pfu DNA Polymerase
(Pyrococcus furiosus)

Mixture of thermostable *Taq* DNA Polymerase, proofreading *Pfu* 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.

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 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 Conditions: Store at –20˚C.



PCR amplification using EURx Perpetual Opti*Taq* DNA Polymerase. Lane M: molecular size marker-Perfect 1 kb DNA Ladder. Lanes 0.5 to 20 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U EURx Perpetual Opti*Taq* DNA Polymerase in 50 µl reaction volume.

An initial denaturation step for 3–5 minutes at 95˚C is recommended to ensure a complete denaturation of the antibody,

Description:

- Perpetual Opti*Taq* DNA Polymerase is a modified and balanced blend containing top quality *Thermus aquaticus* DNA Polymerase, *Pyrococcus furiosus* DNA Polymerase and anti-*Taq* DNA Polymerase antibodies.
- Ultrapure, recombinant enzymes are used to prepare Perpetual Opti*Taq* 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 94-95˚C for two minutes.
- Formation of 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 fast and convenient method when assembling multiple PCR reactions.
- Both increased specificity and reduced mispriming improve multiplex PCR.
- 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 a wide range of PCR products.
- Maintains the 5'→3' exonuclease activity.
- Adds extra A at the 3' ends. Both, TA- and Blunt End cloning are possible.
- 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 templates containing GC-rich regions, palindromes or multiple repeats.
- Increased amplification product yields and purity.
- Ideal for genomic sequencing and mapping by greatly simplifying contig assembly from large amplification products.
- Perpetual Opti*Taq* DNA Polymerase is recommended for 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.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22˚C), 100 mM KCl, 0.5% Tween 20, 0,5% Igepal CA-630, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol.

10 x Reaction Buffer:

10 x Pol Buffer A (optimalization 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 (coloured):
Similar to 10 x buffer B, but additionally enriched with two gel tracking dyes and a gel loading reagent. The buffer enables direct loading of PCR products to agarose gels.

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.

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.*

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Perpetual Opti*Taq* DNA Polymerase
"HOT START" PCR PROTOCOL

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 10x Pol Buffer A or 0 – 7 µl when using 10 x Pol Buffer B or C	1 - 5 mM 1.5 - 5 mM
dNTP mix (5mM 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 Opti *Taq* 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	-

Thermal Cycling Conditions for Products 0.1-10 kb in Size:

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 Larger Than 10 kb in Size:

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 per additional cycle	
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

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 *Taq* DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a non preheated (room temperature) thermal cycler.
4. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x Pol Buffer B or 1 x Pol Buffer C) when using 0.2 mM dNTP (each). In most cases these concentrations 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 directly loaded onto an agarose gel without prior addition of a gel loading buffer. The buffer contains a gel loading reagent as well as two gel tracking dyes (a red and a yellow dye) that separate during electrophoresis. In a 1 % (w/v) agarose gel the red dye migrates at the same rate as a 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 to enzymatic manipulation.
6. 1.25 U of Perpetual Opti *Taq* 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.
7. 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).
8. For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
9. Ensure that 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).
10. Complex genomic DNA should be stored at 2-8˚C. Avoid vortexing the genomic DNA.
11. Use only thin-walled 0.2 ml tubes performing long PCR amplification.

Notes:

1. A 2 min initial denaturation step at 94-95˚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 bp 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 an elongation temperature of 68˚C is strongly recommended.
6. For PCR products exceeding 10 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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