

# Color Perpetual OptiTaq DNA Polymerase

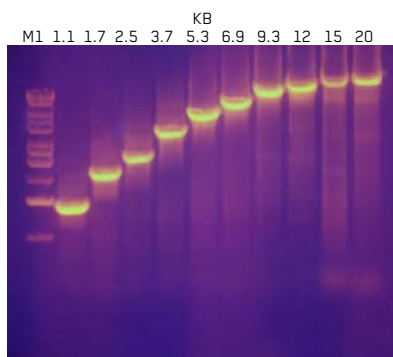
*Color traced monoclonal antibody automatic "Hot Start" PCR system*

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

Cat. No.	Size
E2730-01	200 units
E2730-04	500 units
E2730-02	1000 units
E2730-03	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<sub>2</sub>, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [<sup>3</sup>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 Color Perpetual OptiTaq DNA Polymerase.** Lane M: molecular size marker- Perfect 1 kb DNA Ladder. Lanes 0.5 to 9.3 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U EURx Color Perpetual OptiTaq DNA Polymerase in 50 µl reaction volume; Lanes 12 to 20 kb: PCR amplification reactions, using Pol Buffer C with 0.35 mM dNTPs and 1.25 U EURx Color Perpetual OptiTaq DNA Polymerase in 50 µl reaction volume.

Use of Perpetual Color OptiTaq DNA Polymerase offers several advantages:

- visualizes the addition of the polymerase to the reaction;
- confirms complete mixing;
- serves as tracking dye during gel electrophoresis;
- do not interfere with most downstream applications (exception: is not recommended for any downstream applications using absorbance or fluorescence excitation).

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

**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. The enzyme is supplemented with two inert gel tracking dyes, allowing direct loading of PCR products to agarose gels.**

**Description:**

- Color Perpetual OptiTaq 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 Color 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 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.
- 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 is possible.
- Suitable for multiplex PCR due to increased specificity, wider tolerance for Mg<sup>2+</sup>, 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.
- Color 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 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, 50% glycerol.

**10 x Reaction Buffer:**

**10 x Pol Buffer A (optimization buffer without MgCl<sub>2</sub>):**

The buffer allows to optimize MgCl<sub>2</sub> concentration.

**10 x Pol Buffer B (general application, up to 8-10 kb):**

The buffer contains 15 mM MgCl<sub>2</sub> and is optimized for use with 0.2 mM of each dNTP.

**10 x Pol Buffer C (for products over 8-10 kb):**

The buffer contains 17.5 mM MgCl<sub>2</sub> and is optimized for use with 0.35 mM of each dNTP.

**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.

## Color Perpetual Opti*Taq* DNA Polymerase PCR PROTOCOL

### Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Pol Buffer A or 10 x Pol Buffer B or	5 µl	1x
50 mM MgCl <sub>2</sub>	1-5 µl when using 10 x Pol Buffer A or 0 µl when using 10 x Pol Buffer B	1-5mM  1.5 mM
dNTP mix (5mM each)	2 µl when using 10 x Pol Buffer A or 10 x Pol Buffer B	0.2 mM of each dNTP
Upstream primer	Variable	0.3-0.5 µM
Downstream primer	Variable	0.3-0.5 µM
Color Perpetual Opti <i>Taq</i> DNA Polymerase, 1 U/µl	1.25 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 Color Perpetual Opti*Taq* DNA Polymerase allows room temperature reaction setup.
3. Reactions can be placed in a non preheated (room temperature) thermal cycler.
4. Standard concentrations of MgCl<sub>2</sub> in PCR reaction are: 1.5 mM (as provided in the 1 x Pol Buffer B) when using 0.2 mM dNTP (each) and 1.75 mM (as provided in the 1 x Pol Buffer C) when using 0.35 mM dNTP. In most cases these concentrations will produce satisfactory results. However, in some cases, reaction may be improved by determining optimal concentration of MgCl<sub>2</sub>.
5. Use of Color Perpetual Opti*Taq* DNA Polymerase allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The polymerase contains two gel tracking dyes (a red dye and an 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 Color 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. 1.25 U of Color Perpetual Opti*Taq* 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. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
8. As a general guide for how much template DNA to use, start with a minimum 10<sup>4</sup> 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<sup>11</sup> molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10<sup>9</sup> molecules, 1 µg of human genomic DNA equals 3 x 10<sup>5</sup> 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 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.

### 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<sub>m</sub>. Optimal annealing temperatures may be above or below the estimated T<sub>m</sub>. As a starting point, use an annealing temperature 5°C below T<sub>m</sub>.
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 11<sup>th</sup> cycle) is strongly recommended due to loss of processivity of the enzymes blend.