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Color Perpetual Opti*Taq* PCR Master Mix (2x)

2x Color Perpetual Opti*Taq* PCR Master Mix

Taq DNA Polymerase
Pyrococcus sp. DNA Polymerase

Cat. No.	Size
E2775-01	100 reactions 50 µl each
E2775-02	200 reactions 50 µl each
E2775-03	500 reactions 50 µl each

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 for long- term storage (more than 12 months) or at 4°C for up to 2 months.

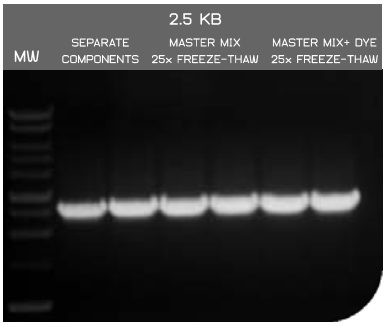

Figure 1: PCR amplification using EURx Color Perpetual Opti*Taq* DNA Polymerase.
A 2.5 kb PCR amplicon was amplified with Perpetual Opti*Taq* DNA Polymerase either in stand-alone or master mix format.
Lane MW: molecular size marker- Perfect 1 kb DNA Ladder (Cat. No. E3130).
Lanes POL (1,2): PCR amplification reactions using 1.25 U Perpetual Opti*Taq* DNA Polymerase, Pol Buffer B and dNTPs
Lanes mM (3,4): PCR amplification reactions using Perpetual Opti*Taq* PCR Master Mix (2x), after 25 freeze-thaw cycles.
Lanes MM+COL (5,6): PCR amplification reactions using Color Perpetual Opti*Taq* PCR Master Mix (2x), after 25 freeze-thaw cycles.

Color Perpetual Opti*Taq* DNA Polymerase Master mix, precolored with two PCR-neutral gel loading dyes. Warrants stable and reproducible high performance even after more than 25 freeze-thaw cycles or more than 12 months of storage.

Description:

- Color Perpetual Opti*Taq* PCR Master Mix (2x) is a ready-to-use solution containing Perpetual Opti*Taq* DNA Polymerase, optimized reaction buffer, $MgCl_2$, dNTPs and two PCR-neutral gel tracking dyes.
- Use of Color Perpetual Opti*Taq* PCR Master Mix (2x) saves time, increases reproducibility (due to minimizing calculation and pipetting errors) and reduces contamination risk (due to fewer pipetting steps) during PCR set-up.
- Perpetual Opti*Taq* DNA Polymerase is a modified and optimized thermostable enzymes blend containing *Thermus aquaticus* DNA polymerase, *Pyrococcus sp.* DNA polymerase and enhancing factors.
- Perpetual Opti*Taq* DNA Polymerase contains recombinant *Taq* DNA Polymerase bound to an anti-*Taq* monoclonal antibody that blocks polymerase activity at moderate temperatures.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated to 94-95°C for two minutes.
- Formation of complexes between *Taq* DNA Polymerase and an anti-*Taq* antibody forms a basis for "hot start" PCR, which allows for convenient room-temperature reaction setup.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to conventional PCR assembly.
- The enzymes blend exhibits 3´→5´ proofreading activity, resulting in considerably higher PCR fidelity, processivity and yield as compared to proofreading-deficient Perpetual *Taq* DNA polymerase (1).
- Ultrapure, recombinant enzymes are used to prepare Color Perpetual Opti*Taq* DNA Polymerase.
- Color Perpetual Opti*Taq* PCR Master Mix is stable with respect to multiple cycles of freezing and thawing. Even after more than 25 freeze-thaw cycles, no decline in performance is detected.
- Same performance as standalone Perpetual Opti*Taq* DNA Polymerase (Cat. No. E2720). Additionally, aliquots of clean nuclease free water are supplied, allowing the setup of PCR reactions without the risk of introducing unwanted DNA through contaminated water.
- Maintains the 5'→3' exonuclease activity.
- Adds an extra A overhang to some, but not all 3'-ends. PCR amplicons are suitable for both, blunt and TA-cloning.
- Color Perpetual Opti*Taq* PCR Master Mix (2x) 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.

Color Perpetual Opti*Taq* PCR Master Mix (2x) Package Contents:

1. Color Perpetual Opti*Taq* PCR Master Mix (2x)
2. Water, nuclease free

Color Perpetual Opti*Taq* PCR Master Mix (2x):

Supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$ and 0.4 mM of each dNTP. Final concentrations: 1.5 mM $MgCl_2$ and 0.2 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. et al. (1996) Nucleic Acid Res. 24 (18) 3546-3551.
2. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I.(1980) Biokhimiya 45, 644.

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PCR PROTOCOL (1)

PCR Reaction Assembly

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
Color Perpetual Opti*Taq* PCR master Mix (2 x)	25 µl	1.25 U Perpetual Opti*Taq* DNA Polymerase 1 x Reaction Buffer (1.5 mM $MgCl_2$) 0.2 mM of each dNTP
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 µM
Template DNA	Variable	0.5 µg/50 µl
Sterile double-distilled water	To 50 µl	-
Total volume	50 µl	-

General formula for calculating total gene / genome copy numbers from the total amount of template DNA:

$$Template\ DNA\ copy\ number\ [molecules] =$$

$$\frac{DNA\ amount\ [ng] \cdot 6.022 x 10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

Optimum: 10^4 template DNA copies
Maximum: 0.5 µg template DNA or less

(MW pro bp: siehe Dolezel et al. Cytometry, 2003, Vol. 51A, 2, 127-8)

Notes

1. **First Law of PCR:** PCR is a sort of homeopathic process. It works best, as long as *all* components are assembled in homeopathic doses only.
2. **Concentration Differences.** Thaw, gently vortex and centrifuge Color Perpetual Opti*Taq* PCR Master Mix (2x) and primers to avoid localized differences in salt concentration.
3. **Room Temperature:** Set up PCR reactions at room temperature. Use of Color Perpetual Opti*Taq* PCR Master Mix (2x) allows room temperature reaction setup.
4. **Primer Mix:** Primers can be added separately or as a primer mix prepared previously.
5. **Mix Template:** Vortex the samples and briefly spin down.
6. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler.
7. **$MgCl_2$.** Standard concentration of $MgCl_2$ in PCR reactions is 1.5 mM (as provided in the 1 x Color Perpetual Opti*Taq* Master Mix) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, if higher $MgCl_2$ concentrations are required, prepare a 25 mM $MgCl_2$ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 50 µl will add 25 nmol $MgCl_2$ and thus increase total Mg^{2+} reaction concentration in 0.5 mM.
8. **Colored enzyme mix.** Use of Color Perpetual Opti*Taq* PCR Master Mix (2x) allows PCR reactions to be loaded directly onto agarose gels without prior addition of a gel loading buffer. The master mix contains two gel tracking dyes (a red dye and an yellow dye), which separate during electrophoresis. On a 1 % [w/v] agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment whereas 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 downstream enzymatic manipulation.
9. **PCR additives / PCR Enhancers.** In most cases there is no need to add any 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% [v/v]. The recommended starting DMSO concentration (if required) is 3% [v/v].
10. **Template Copies.** 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 9.8 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).
General formula for calculating total gene /genome copy number from template DNA mass:
copy number [molecules] = (DNA amount [ng] x 6.022 x 10^{23} [molecules mol^{-1}]) / (length [bp] x 1x10^9 [ng g^{-1}] x 616 [g bp^{-1}])
(MW per bp: see Dolezel et al. Cytometry, 2003, Vol. 51A, 2, 127-8)
11. **Template DNA for Long Range PCR.** For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
12. **High Quality Template DNA:** 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).
13. **Storage of High Molecular Weight DNA:** Complex genomic DNA should be stored at 2-8°C. Avoid vortexing genomic DNA.
14. **Thin Walled Reaction Cups:** Use only thin-walled 0.2 ml tubes performing long PCR amplification.

Color Perpetual OptiTaq PCR Master Mix (2x)

PCR PROTOCOL (2)

Thermal Cycling Conditions

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

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	93-95˚C	2-5 min	1
Denaturation Annealing Extension	93-95˚C 50-68˚C 72˚C or 68˚C	15-30 s 30 s 1 min/1 kb	25-35
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	Tempera-ture	Time	Number of Cycles
Initial Denaturation	92-94˚C	2 min	1
Denaturation Annealing Extension	92-94˚C 60-68˚C 68˚C	10-15 s 30 s 1 min/1 kb	10
Denaturation Annealing Extension	92-94˚C 60-68˚C 68˚C	10-15 s 30 s 1 min/1 kb +20 s per additional cycle	15-25
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Notes

1. **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.
2. **Long PCR – Primer Requirements:** 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.
3. **Long PCR – Short Denaturation Steps:** 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.
4. **Long PCR – Low Elongation Temperature:** For PCR products exceeding 5 kb in size, an elongation temperature of 68˚C is strongly recommended.
5. **Long PCR – Extended Elongation Period:** 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.
6. **Minimize Time for Cooling Step (+4˚C).** Overly prolonged cooling time to 4˚C puts a hard strain on your PCR cycler's Peltier elements. To prevent an all too early wearout, minimize the time for the last cooling step at 4˚C and, upon completion of the PCR reaction, remove PCR samples from the cycler as soon as possible.
   Alternately, choose +8˚C as final cooling temperature, but take care to remove the PCR samples as soon as possible for minimizing PCR amplicon exposure time to 3'-5' exonuclease activity of *Pyrococcus sp.* DNA polymerase.