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Color Perpetual *Taq* DNA Master Mix (2x)

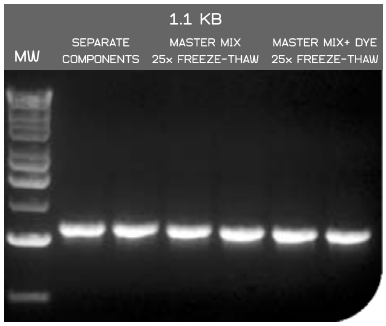
Monoclonal antibody automatic "Hot Start" PCR system

Taq DNA Polymerase
(Thermus aquaticus)

Cat. No.	Size
E2745-01	100 reactions 50 µl each
E2745-02	200 reactions 50 µl each
E2745-03	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.



PCR amplification using EURx Color Perpetual *Taq* PCR Master Mix (2x).
A 1.1 kb amplicon of the human CCR5 gene was amplified with Perpetual *Taq* DNA Polymerase in stand-alone or master mix formats.
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 *Taq* DNA Polymerase, Pol Buffer B and dNTPs
Lanes MM (3,4): PCR amplification reactions using Perpetual *Taq* PCR Master Mix (2x), after 25 freeze-thaw cycles
Lanes MM+COL (5,6): PCR amplification reactions using Color Perpetual *Taq* PCR Master Mix (2x) after 25 freeze-thaw cycles

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

Color Perpetual *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. Pre-complexed with specific anti-*Taq* monoclonal antibody for automatic "hot start" PCR.

Description:

- Color Perpetual Taq PCR Master Mix (2x) is a ready-to-use solution containing Perpetual *Taq* DNA Polymerase, optimized reaction buffer, $MgCl_2$, dNTPs and two gel tracking dyes.
- Use of Color Perpetual *Taq* PCR Master Mix (2x) saves time, increases reproducibility (due to avoiding calculation and pipetting errors) and reduces contamination risk (due to fewer pipetting steps) during PCR set-up.
- Color Perpetual *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 *Taq* DNA Polymerase (Cat. No. E2500). 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.
- Perpetual *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 the conventional PCR assembly method.
- Perpetual *Taq* DNA Polymerase replicates DNA at 72˚C and exhibits a half-life of 40 min at 95˚C (1,2).
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at 3' ends.
- Color Perpetual *Taq* 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 10 kb.

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

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

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

Perpetual *Taq* DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$, 0.4 mM of each dNTP and two PCR-neutral gel tracking dyes.
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. *Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550.*
2. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I.(1980) Biokhimiya 45, 644.*

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
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Color Perpetual *Taq* PCR Master Mix (2x)

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
Color Perpetual *Taq* PCR Master Mix (2x)	25 µl	1.25 U *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 per 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 briefly centrifuge Color Perpetual *Taq* PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
3. **Room Temperature.** Set up PCR reactions at room temperature. Use of Color Perpetual *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 reaction is 1.5 mM (as provided with the 1 x Color Perpetual *Taq* PCR 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 *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. **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 9.1 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).

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation	94-95°C	15-60 s	25-35
Annealing	50-68°C	30-60 s	
Extension	72°C	1 min/1 kb	
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. **Initial Denaturation.** A 2 min initial denaturation step at 94-95°C is required to inactivate the antibody and restore the polymerase activity.
2. **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.
3. **Long PCR.** When amplifying long PCR products (longer than 5 kb):
   a. initial denaturation should be 2 min at 94°C
   b. cycle denaturation should be 15-20 s at 94°C
   c. use an elongation temperature of 68°C instead of 72°C.

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