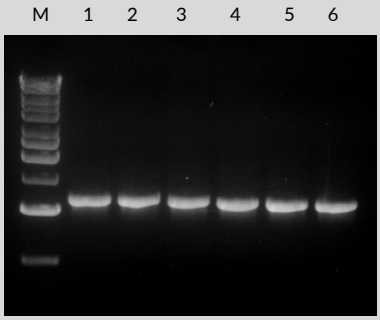
EURx® MOLECULAR BIOLOGY PRODUCTS

Color Perpetual Taq PCR Master Mix (2x)

Cat. No.	size
E2745-01	100 reactions of 50 µl
E2745-02	200 reactions of 50 µl
E2745-03	500 reactions of 50 µl

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 for long-term storage 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 M: molecular size marker- Perfect 1 kb DNA Ladder.

Lanes 1, 2: PCR amplification reactions using 1.25 U Color Perpetual Taq DNA Polymerase, Pol Buffer B and dNTPs.

Lanes 3, 4: PCR amplification reactions using Perpetual Taq PCR Master Mix (2x), after 25 freeze -thaw cycles.

Lanes 5, 6: PCR amplification reactions using Color Perpetual Taq PCR Master Mix (2x), after 25 freeze -thaw cycles.

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.*

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) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR setup.
- Color Perpetual Taq DNA Polymerase contains recombinant Taq DNA Polymerase bound to an anti-Taq monoclonal antibody that blocks polymerase activity at moderate temperatures.
- Anti-Taq antibodies inhibit polymerase activity at temperatures up 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 "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.
- Color 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 the 3' ends.
- Color Perpetual Taq 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 10 kb.

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

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

Color Perpetual Taq PCR Master Mix (2x):

Color 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 gel tracking dyes.

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
Color Perpetual Taq PCR Master Mix (2x)	25 µl	1.25 U Perpetual 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	-

Thermal Cycling Conditions:

Step	Temperature	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. Thaw, gently vortex and briefly centrifuge Color Perpetual Taq PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. Set up PCR reactions at room temperature. Use of Color Perpetual Taq PCR Master Mix (2x) allows room temperature reaction setup.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Reactions can be placed in a room temperature thermal cycler.
6. 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 a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to a reaction.
7. Use of Color Perpetual Taq PCR Master Mix (2x) allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The master mix 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.
8. 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%.
9. 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).

Notes:

1. 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. When amplifying long PCR products (over 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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