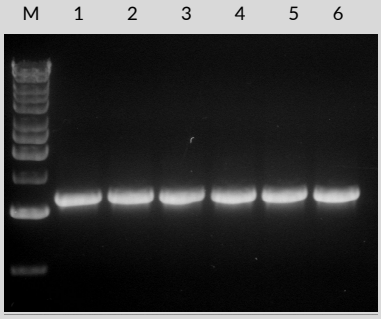
EURx® MOLECULAR BIOLOGY PRODUCTS

Color Taq PCR Master Mix (2x)

Cat. No.	size
E2525-01	100 reactions of 50 µl
E2525-02	200 reactions of 50 µl
E2525-03	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 Taq PCR Master Mix (2x). A 1.1 kb amplicon of the human CCR5 gene was amplified with 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 Taq DNA Polymerase, Pol Buffer B and dNTPs
Lanes 3, 4: PCR amplification reactions using Taq PCR Master Mix (2x), after 25 freeze-thaw cycles
Lanes 5, 6: PCR amplification reactions using Color 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 Taq PCR Master Mix (2x) is a ready-to-use solution containing Taq DNA Polymerase, optimized reaction buffer, $MgCl_2$, dNTPs and two gel tracking dyes.
- Use of Color Taq PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR set-up.
- Taq DNA Polymerase is a thermostable enzyme of approximately 94 kDa from *Thermus aquaticus.*
- Ultrapure, recombinant protein.
- The enzyme replicates DNA at 74°C and exhibits a half-life of 40 min at 95°C (1,2).
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Maintains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.
- Color 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 10 kb.

Color Taq PCR Master Mix (2x) contains:

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

Color Taq PCR Master Mix (2x):

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 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	-

Notes:

1. Thaw, gently vortex and briefly centrifuge Color Taq PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. PCR reactions should be set up on ice.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Place reactions in a thermal cycler that has been preheated to 94-95°C.
6. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided with the 1 x Color 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 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).

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. 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. 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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