MANUFACTURED BY EURx

roboklon

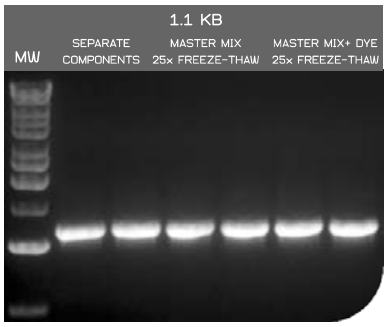
Color *Taq* PCR Master Mix (2x)

2x *Color Taq* PCR Master Mix
Taq DNA Polymerase

Cat. No.	Size
E2525-01	100 reactions 50 µl each
E2525-02	200 reactions 50 µl each
E2525-03	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 *Taq* DNA Master Mix.
A 1.1 kb amplicon of the human CCR5 gene was amplified with *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 *Taq* DNA Polymerase, Pol Buffer B and dNTPs
Lanes mM (3,4): PCR amplification reactions using *Taq* PCR Master Mix (2x) (no gel loading dyes), after 25 freeze-thaw cycles.
Lanes MM+COL (5,6): PCR amplification reactions using Color Taq PCR Master Mix (2x) (with gel loading dyes) after 25 freeze-thaw cycles.

Color *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 *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) saves time, increases reproducibility (due to minimizing calculation and pipetting errors) and reduces contamination risk (due to fewer pipetting steps) during PCR set-up.
- Color *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.
- *Taq* DNA Polymerase is a thermostable enzyme of approximately 94 kDa from *Thermus aquaticus.*
- Ultra pure, 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.
- *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) Package Contents:

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

Color *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. Contains 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.

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.*

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Color *Taq* PCR Master Mix (2x)

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
Color *Taq* PCR master Mix (2 x)	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)

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. **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 *Taq* PCR Master Mix (2x) and primers to avoid localized differences in salt concentration.
3. **On Ice.** PCR reactions should be set up on ice.
4. **Primer Mix.** Primers can be either added separately or as primer mix prepared separately.
5. **Mix Template.** Vortex the samples and briefly spin down.
6. **Preheat Cycler.** Place reactions in a thermal cycler that has been preheated to 94-95°C.
7. **$MgCl_2$.** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x Color *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 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 9.8 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).

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.** 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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