Taq PCR Master Mix (2x)

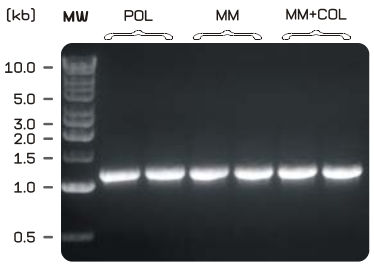
2x *Taq* PCR Master Mix

Taq DNA Polymerase

Cat. No.	Size
E2520-01	100 reactions 50 µl each
E2520-02	200 reactions 50 µl each
E2520-03	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 *Taq* DNA Polymerase.
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), after 25 freeze-thaw cycles.
Lanes MM+COL (5,6): PCR amplification reactions using Taq PCR Master Mix (2x) and 10 x Color Load, after 25 freeze-thaw cycles.

***Taq* DNA Polymerase Master mix, with stable and reproducible high performance even after more than 25 freeze-thaw cycles or more than 12 months of storage.**

Description:

- *Taq* PCR Master Mix (2x) is a ready-to-use solution containing *Taq* DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of *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.
- *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 *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.
- For optional use, a 10 x Color load buffer is supplied. The Color Load buffer allows to directly load PCR products to agarose gels.
- *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.

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

1. *Taq* PCR Master Mix (2x)
2. Water, nuclease free
3. 10 x Color Load Buffer

***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.

10 x Color Load:

10 x Color Load contains two gel tracking dyes and a gel loading reagent. It enables direct loading of PCR products onto an agarose gel.

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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PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
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
Optional: 10 x Color Load	5 µl	1 x
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	To 50 µl	-
Total volume	50 µl	-

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. **Concentration differences:** Thaw, gently vortex and centrifuge *Taq* PCR Master Mix (2x) and primers to avoid localized differences in salt concentration.
2. **On Ice**: PCR reactions should be set up on ice.
3. **Primer Mix:** Primers can be either added separately or as primer mix prepared separately.
4. **Mix Template:** Vortex the samples and briefly spin down.
5. **Preheat Cycler:** Place reactions in a thermal cycler that has been preheated to 94-95°C.
6. **$MgCl_2$:** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x *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 $MgCl_2$ reaction concentration in 0.5 mM.
7. **Color Load:** Use of the 10 x Color Load allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer.The 10 x Color Load contains a gel loading reagent and two gel tracking dyes (a red dye and an 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 to enzymatic manipulation.
8. **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)

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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