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

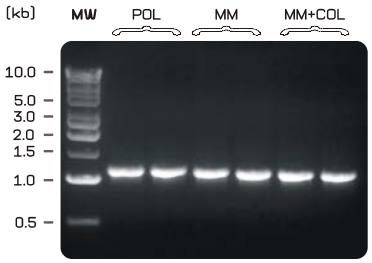
*Monoclonal antibody automatic "Hot Start" PCR system*

## *Taq* DNA Polymerase
*(Thermus aquaticus)*

| Cat. No. | Size |
|---|---|
| E2740-01 | 100 reactions 50 µl each |
| E2740-02 | 200 reactions 50 µl each |
| E2740-03 | 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 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 Perpetual *Taq* PCR Master Mix (2x) and 10 x Color Load, 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,**

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

**Description:**

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

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

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

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

Perpetual *Taq* DNA Polymerase is 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 agarose gels.

**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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# Perpetual *Taq* PCR Master Mix (2x)

## PCR PROTOCOL

**Preparation of PCR Reaction:**

| Component | Volume / Reaction | Final Concentration |
|---|---|---|
| Perpetual *Taq* PCR Master Mix (2x) | 25 µl | 1.25 U *Taq* DNA Polymerase<br>1 x Reaction Buffer (1.5 mM $MgCl_2$)<br>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<br>Annealing<br>Extension | 94-95˚C<br>50-68˚C<br>72˚C | 15-60 s<br>30-60 s<br>1 min/1 kb | 25-35 |
| Final Extension | 72˚C | 7 min | 1 |
| Cooling | 4˚C | Indefinite | 1 |

**Notes:**

1. **Concentration Differences:** Thaw, gently vortex and briefly centrifuge Perpetual *Taq* PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. **Room Temperature:** Set up PCR reactions at room temperature. Use of Perpetual *Taq* PCR Master Mix (2x) allows room temperature reaction setup.
3. **Primer Mix:** Primers can be added separately or as a primer mix prepared previously.
4. **Mix Template:** Vortex the samples and briefly spin down.
5. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler.
6. **$MgCl_2$:** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided with the 1 x 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 $MgCl_2$ 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 104 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 1011 molecules, 1 µg of E. coli genomic DNA equals 2 x 108 molecules, 1 µg of human genomic DNA equals 3 x 105 molecules).
   General formula for calculating total gene /genome copy number from template DNA mass:
   copy number [molecules] = (DNA amount [ng] x 6.022 x 1023 [molecules mol-1]) / (length [bp] x 1x109 [ng g-1] x 616 [g bp-1])
   (MW per bp: see Dolezel et al. Cytometry, 2003, Vol. 51A, 2, 127-8)

**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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