

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

*Monoclonal antibody automatic "Hot Start" PCR system*

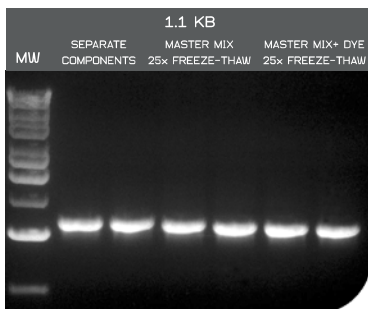
### dUTP Perpetual *Taq* Master Mix (2x) (*Thermus aquaticus*)

Cat. No.	Size
E2741-01	100 reactions 50 µl each
E2741-02	200 reactions 50 µl each
E2741-03	1000 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<sub>2</sub>, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [<sup>3</sup>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 EUR<sub>x</sub> 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<sub>2</sub> 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.
- dUTP Perpetual *Taq* Master Mix (2x) contains dUTP, which partially replaces dTTP. It allows the optional use of a uracil N-glycosylase (UNG) to prevent carryover contamination between reactions.
- 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
4. Thermolabile Uracil N-Glycosylase (UNG)

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

Perpetual *Taq* DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM MgCl<sub>2</sub> and 0.4 mM of each dNTP.

dTTP is partially replaced by dUTP.

Final concentrations: 1.5 mM MgCl<sub>2</sub> and 0.2 mM of each dNTP.

#### 10 x Color Load:

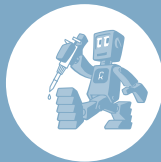
10 x Color Load contains two gel tracking dyes and a gel loading reagent. It enables direct loading of PCR products onto agarose gels.

#### 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.
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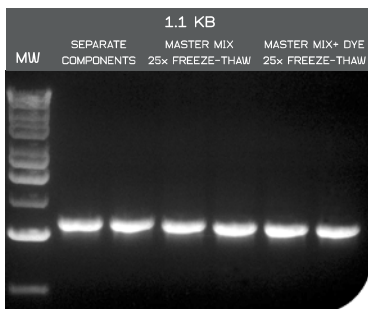
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