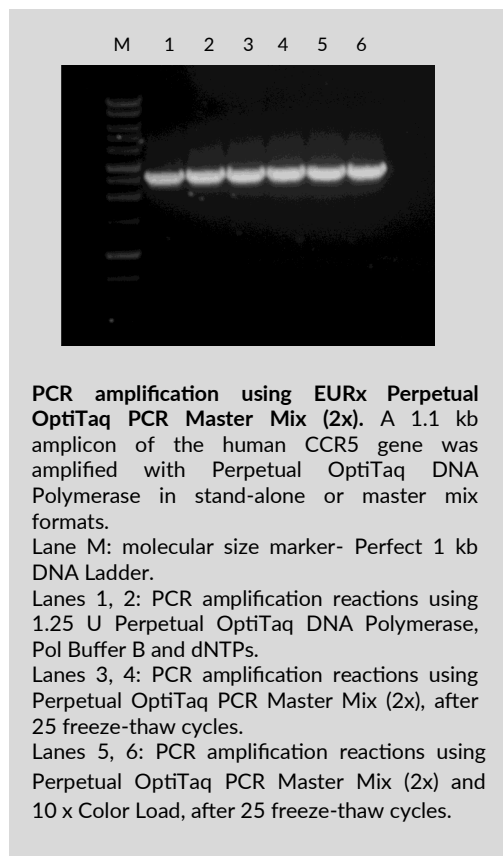


Perpetual OptiTaq PCR Master Mix (2x)

Cat. No.	size
E2770-01	100 reactions of 50 µl
E2770-02	200 reactions of 50 µl
E2770-03	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 MgCl₂, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [³H]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 EUR_x Perpetual OptiTaq PCR Master Mix (2x). A 1.1 kb amplicon of the human CCR5 gene was amplified with Perpetual OptiTaq 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 Perpetual OptiTaq DNA Polymerase, Pol Buffer B and dNTPs.

Lanes 3, 4: PCR amplification reactions using Perpetual OptiTaq PCR Master Mix (2x), after 25 freeze-thaw cycles.

Lanes 5, 6: PCR amplification reactions using Perpetual OptiTaq PCR Master Mix (2x) and 10 x Color Load, after 25 freeze-thaw cycles.

Description:

- Perpetual OptiTaq PCR Master Mix (2x) is a ready-to-use solution containing Perpetual OptiTaq DNA Polymerase, optimized reaction buffer, MgCl₂ and dNTPs.
- Use of Perpetual OptiTaq PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR set-up.
- Perpetual OptiTaq DNA Polymerase is a modified and balanced blend containing top quality *Thermus aquaticus* DNA Polymerase, *Pyrococcus sp.* DNA Polymerase and anti-Taq DNA Polymerase antibodies.
- 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 at 92-95°C for two minutes.
- Formation of inactive complexes between Taq DNA Polymerase and an anti-Taq antibody forms a basis for automatic "hot start" PCR, which allows for the assembly of PCR reactions at room temperature.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- The enzymes blend exhibits the 3'→5' proofreading activity, resulting in considerably higher PCR fidelity and processivity than possible with unmodified Taq DNA polymerase.
- Enables increased amplification product yield in comparison with Taq DNA polymerase over wide range of PCR products.
- Maintains the 5'→3' exonuclease activity.
- Adds extra A at the 3' ends.
- Perpetual OptiTaq 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 20 kb.

Perpetual OptiTaq PCR Master Mix (2x) contains:

- Perpetual OptiTaq PCR Master Mix (2x)
- Water, nuclease free
- 10 x Color Load

Perpetual OptiTaq PCR Master Mix (2x):

Perpetual OptiTaq DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM MgCl₂, 0.4 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.

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
Perpetual OptiTaQ PCR Master Mix (2x)	25 µl	1.25 U Perpetual OptiTaQ 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	-

Notes:

1. Thaw, gently vortex and briefly centrifuge Perpetual OptiTaQ PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. Set up PCR reactions at room temperature. Use of Perpetual OptiTaQ PCR Master Mix (2x) allows room temperature reaction setup.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Reactions can be placed in a room temperature thermal cycler.
6. Standard concentration of MgCl₂ in PCR reaction is 1.5 mM (as provided with the 1 x Perpetual OptiTaQ PCR Master Mix) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, if a higher MgCl₂ concentration is required, prepare a 25 mM MgCl₂ stock solution and add to a reaction.
7. 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 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 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).
10. For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
11. Ensure that a template DNA is of sufficiently high quality. Use only high-molecular-weight DNA, when amplifying long PCR targets (over 20-50 kb, depending on the amplicon length).
12. Complex genomic DNA should be stored at 2-8°C. Avoid vortexing the genomic DNA.
13. Use only thin-walled 0.2 ml tubes performing long PCR amplification.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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Thermal Cycling Conditions for Products 0.1-10 kb:

Step	Temperature	Time	Numbers of Cycles
Initial Denaturation	93-95°C	2-5 min	1
Denaturation	93-95°C	15-30 s	
Annealing	50-68°C	30 s	25-35
Extension	72°C or 68°C	1 min/1 kb	
Final Extension	72°C or 68°C	7 min	1
Cooling	4°C	Indefinite	1

Thermal Cycling Conditions for Products over 10 kb:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	92-94°C	2 min	1
Denaturation	92-94°C	10-15 s	10
Annealing	60-68°C	30 s	
Extension	68°C	1 min/1 kb	
Denaturation	92-94°C	10-15 s	15-25
Annealing	60-68°C	30 s	
Extension	68°C	1 min/1 kb +20 s in each	
Final Extension	68°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. Typical primers for long PCR amplification have a length of 22-34 and should have annealing temperatures above 60°C to enhance reaction specificity.
3. When amplifying long PCR products keep denaturation steps as short as possible and denaturation temperature as low as possible (try not to exceed 2 min at 94°C during initial denaturation and 10-15 s at 94°C during cycle denaturation step). Sometimes fragments with high GC-content need higher denaturation temperatures, but keep in mind that the yield increases when denaturation temperature/duration is decreased.
4. For PCR products over 5 kb elongation temperature of 68°C is strongly recommended.
5. For PCR products over 10 kb elongation of extension step (+20 s in each additional cycle starting from 11th cycle) is strongly recommended due to loss of processivity of the enzymes blend.

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