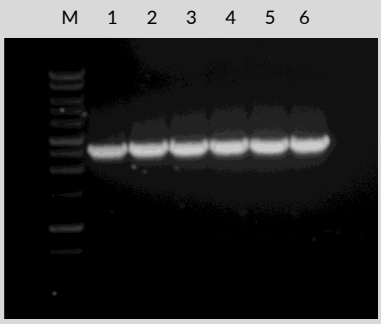
EURx® MOLECULAR BIOLOGY PRODUCTS

Color OptiTaq PCR Master Mix (2x)

Cat. No.	size
E2915-01	100 reactions of 50 µl
E2915-02	200 reactions of 50 µl
E2915-03	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 $MgCl_2$, 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 or at 4°C for up to 2 months.



PCR amplification using EURx Color OptiTaq PCR Master Mix (2x). A 2.5 kb amplicon of the human CCR5 gene was amplified with 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 Color OptiTaq DNA Polymerase, Pol Buffer B and dNTPs.
Lanes 3, 4: PCR amplification reactions using OptiTaq PCR Master Mix (2x), after 25 freeze-thaw cycles.
Lanes 5, 6: PCR amplification reactions using Color OptiTaq PCR Master Mix (2x), after 25 freeze-thaw cycles.

Description:

- Color OptiTaq PCR Master Mix (2x) is a ready-to-use solution containing OptiTaq DNA Polymerase, optimized reaction buffer, $MgCl_2$, dNTPs and two gel tracking dyes.
- Use of Color OptiTaq PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR set-up.
- OptiTaq DNA Polymerase is a modified and optimized thermostable enzymes blend containing *Thermus aquaticus* DNA polymerase and *Pyrococcus sp.* DNA polymerase.
- Ultrapure, recombinant enzymes are used to prepare OptiTaq DNA Polymerase.
- 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.
- Color 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.

Color OptiTaq PCR Master Mix (2x) contains:

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

Color OptiTaq PCR Master Mix (2x):

OptiTaq DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$, 0.4 mM of each dNTP and 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.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
Color OptiTaq PCR Master Mix (2x)	25 µl	1.25 U 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
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 Color OptiTaq PCR Master Mix (2x) and primers before use to avoid localized differences in salt concentration.
2. PCR reactions should be set up on ice.
3. Primers can be added separately or as a primer mix prepared previously.
4. Vortex the samples and briefly spin down.
5. Place reactions in a thermal cycler that has been preheated to 94-95°C.
6. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided with the 1 x Color OptiTaq PCR Master Mix) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to a reaction.
7. Use of Color OptiTaq PCR Master Mix (2x) allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The Master Mix 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 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 9.1 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).
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.

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Thermal Cycling Conditions for Products 0.1-10 kb:

Step	Temperature	Time	Numbers of
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 additional cycle	
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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