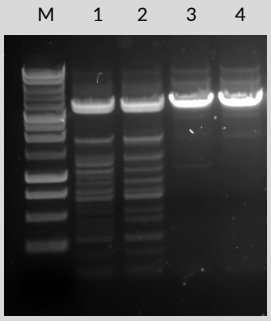
EURx® MOLECULAR BIOLOGY PRODUCTS

tiOptiTaq PCR Master Mix (2x)

Cat. No.	size
E2726-01	100 reactions of 50 µl
E2726-02	200 reactions of 50 µl
E2726-03	500 reactions of 50 µl

Storage Conditions: Store at -20°C.



PCR amplification using EURx tiOptiTaq PCR Master Mix (2x). A 4 kb amplicon of the human β-globin gene was amplified with tiOptiTaq PCR Master Mix (2x).

Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder.

Lanes 1, 2: PCR amplification reactions using OptiTaq PCR Master Mix (2x). Reactions were incubated 30 min at 25°C before PCR.

Lanes 3, 4: PCR amplification reactions using tiOptiTaq PCR Master Mix (2x). Reactions were incubated 30 min at 25°C before PCR.

Description:

- tiOptiTaq PCR Master Mix (2x) is a ready-to-use solution containing tiOptiTaq DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of tiOptiTaq PCR Master Mix (2x) allows to save time and reduce contamination risk due to fewer pipetting steps during PCR set-up.
- tiOptiTaq DNA Polymerase is a new generation „hot start" enzyme blend that is blocked at moderate temperatures and allows room temperature reactions setup.
- The polymerase activity is restored during normal cycling conditions.
- Use of tiOptiTaq DNA Polymerase allows for the enormous increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- tiOptiTaq DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions and 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.
- tiOptiTaq 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.

tiOptiTaq PCR Master Mix (2x) contains:

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

tiOptiTaq PCR Master Mix (2x):

tiOptiTaq DNA Polymerase is supplied in 2 x Pol Buffer B containing 3 mM $MgCl_2$, 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.

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Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
tiOptiTaq PCR Master Mix (2x)	25 µl	1.25 U tiOptiTaq 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 tiOptiTaq 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 tiOptiTaq 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 $MgCl_2$ in PCR reaction is 1.5 mM (as provided with the 1 x tiOptiTaq 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 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 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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