MANUFACTURED BY EURx

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TI Opti*Taq* PCR Master Mix (2x)

2x TI Opti*Taq* PCR Master Mix

Taq DNA Polymerase

Pyrococcus sp. DNA Polymerase

Cat. No.	Size
E2726-01	100 reactions 50 µl each
E2726-02	200 reactions 50 µl each
E2726-03	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.

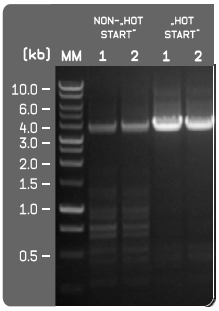

Figure 1: PCR amplification using EURx *TI* Opti*Taq* Master Mix.
PCR amplification using EURx tiOptiTaq PCR Master Mix (2x). A 4 kb amplicon of the human beta-globin gene was amplified with tiOptiTaq PCR Master Mix (2x). To demonstrate the impact of automated "HotStart" on preventing PCR artifact formation due to unspecific annealing, PCR reactions were incubated 30 min at 25°C before PCR.
Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder (Cat. No. E3131).
Lanes Non-"HotStart" 1, 2: PCR amplification reactions using non-"Hot Start" OptiTaq PCR Master Mix (2x).
Lanes "HotStart" 1, 2: PCR amplification reactions using tiOptiTaq PCR Master Mix (2x).

TI Opti*Taq* DNA Polymerase master mix, with stable and reproducible high performance.

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. DNA Polymerase activity is restored during normal PCR 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 3' 5' proofreading activity, resulting in considerably higher PCR fidelity and processivity as compared to non-modified Taq DNA polymerase.
- Enables increased amplification product yield in comparison to Taq DNA polymerase over wide range of PCR products.
- Maintains the 5' 3' exonuclease activity.
- Adds extra A to 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.

***Taq* PCR Master Mix (2x) Package Contents:**

1. TI Opti*Taq* PCR Master Mix (2x)
2. Water, nuclease free
3. 10 x Color Load Buffer

TI Opti*Taq* PCR Master Mix (2x):

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 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.

References:

1. Cline J. et al. (1996) Nucleic Acid Res. 24 (18) 3546-3551.

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TI Opti*Taq* PCR Master Mix (2x)

PCR PROTOCOL (1)

PCR Reaction Assembly

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
TI Opti*Taq* PCR master Mix (2 x)	25 µl	1.25 U TI Opti*Taq* 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	-

General formula for calculating total gene / genome copy numbers from the total amount of template DNA:

$$Template\ DNA\ copy\ number\ [molecules] =$$

$$\frac{DNA\ amount\ [ng] \cdot 6.022 x 10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

Optimum: 10^4 template DNA copies
Maximum: 0.5 µg template DNA or less

(MW pro bp: siehe Dolezel et al. Cytometry, 2003, Vol. 51A, 2, 127-8)

Notes

1. **First Law of PCR:** PCR is a sort of homeopathic process. It works best, as long as *all* components are assembled in homeopathic doses only.
2. **Concentration Differences.** Thaw, gently vortex and centrifuge TI Opti*Taq* PCR Master Mix (2x) and primers to avoid localized differences in salt concentration.
3. **Room Temperature:** Set up PCR reactions at room temperature. Use of TI Opti*Taq* PCR Master Mix (2x) allows room temperature reaction setup.
4. **Primer Mix:** Primers can be added separately or as a primer mix prepared previously.
5. **Mix Template:** Vortex the samples and briefly spin down.
6. **No Preheating Required:** Reactions can be placed in a room temperature thermal cycler.
7. **$MgCl_2$.** Standard concentration of $MgCl_2$ in PCR reactions is 1.5 mM (as provided in the 1 x TI Opti*Taq* 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.
8. **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.
9. **PCR additives / PCR Enhancers.** In most cases there is no need to add any 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% [v/v]. The recommended starting DMSO concentration (if required) is 3% [v/v].
10. **Template Copies.** 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 9.8 x 10^{11} molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10^6 molecules,1 µg of human genomic DNA equals 3 x 10^5 molecules).
General formula for calculating total gene /genome copy number from template DNA mass:
copy number [molecules] = (DNA amount [ng] x 6.022 x 10^{23} [molecules mol^{-1}]) / (length [bp] x 1x10^9 [ng g^{-1}] x 616 [g bp^{-1}])
(MW per bp: see Dolezel et al. Cytometry, 2003, Vol. 51A, 2, 127-8)
11. **Template DNA for Long Range PCR.** For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
12. **High Quality Template DNA:** Ensure that 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).
13. **Storage of High Molecular Weight DNA:** Complex genomic DNA should be stored at 2-8°C. Avoid vortexing genomic DNA.
14. **Thin Walled Reaction Cups:** Use only thin-walled 0.2 ml tubes performing long PCR amplification.

TI OptiTaq

PCR Master Mix (2x)

PCR PROTOCOL (2)

Thermal Cycling Conditions

Thermal Cycling Conditions for Products 0.1-10 kb in Size:

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

Thermal Cycling Conditions for Products Larger Than 10 kb in Size:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	92-94˚C	2 min	1
Denaturation Annealing Extension	92-94˚C 60-68˚C 68˚C	10-15 s 30 s 1 min/1 kb	10
Denaturation Annealing Extension	92-94˚C 60-68˚C 68˚C	10-15 s 30 s 1 min/1 kb +20 s per additional cycle	15-25
Final Extension	68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Notes

1. **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.
2. **Long PCR – Primer Requirements:** Typical primers for long PCR amplification have a length of 22-34 bp and should have annealing temperatures above 60˚C to enhance reaction specificity.
3. **Long PCR – Short Denaturation Steps:** 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. **Long PCR – Low Elongation Temperature:** For PCR products exceeding 5 kb in size, an elongation temperature of 68˚C is strongly recommended.
5. **Long PCR – Extended Elongation Period:** For PCR products exceeding 10 kb in length, an elongation of the extension step (+20 s in each additional cycle, starting from the 11th cycle) is strongly recommended due to loss of processivity of the enzymes blend.
6. **Minimize Time for Cooling Step (+4˚C).** Overly prolonged cooling time to 4˚C puts a hard strain on your PCR cycler's Peltier elements. To prevent an all too early wearout, minimize the time for the last cooling step at 4˚C and, upon completion of the PCR reaction, remove PCR samples from the cycler as soon as possible.
   Alternately, choose +8˚C as final cooling temperature, but take care to remove the PCR samples as soon as possible for minimizing PCR amplicon exposure time to 3'-5' exonuclease activity of *Pyrococcus sp.* DNA polymerase.