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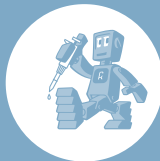


roboklon

TITAQ

ti*Taq* Master Mix

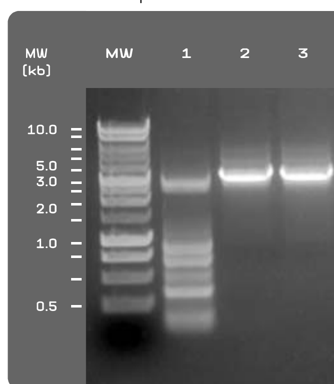
Automatic "Hot Start" PCR system

ti*Taq* Master Mix (2x)

(Thermus aquaticus)

Cat. No.	Size
E2716-01	100 reactions
E2716-02	200 reactions
E2716-03	500 reactions

Storage Conditions: Store at -20°C for long term storage, or at +4°C for up to two months.



PCR amplification using EURx ti*Taq* PCR Master Mix. A 2 kb amplicon of the human beta-globin gene was amplified using tiTaq PCR Master Mix (2x).
Lane M: molecular size marker- Perfect Plus 1 kb DNA Ladder (E3131).
Lane 1: PCR amplification reaction using (non-"Hot Start") Taq PCR Master Mix (2x). Reaction was incubated 30 min at 25°C before PCR.
Lanes 2, 3: PCR amplification reactions using tiTaq PCR Master Mix (2x). Reactions were incubated 30 min at 25°C before PCR.

Enzyme Properties:

Property	Value
Template	ssDNA, dsDNA
5'-3' exonuclease	yes
3'-5' exonuclease	no
Proofreading	no
Strand displacement	no
Error rate	$>10^{-6}$
Relative Accuracy Ratio* (*Taq* = 1)	1
Half life at 95°C	40 min
Amplicon length	up to 10 kb
Generation of 3'-A-overhangs	yes (to a fraction of amplicons)
TA- / blunt cloning possible	yes / yes

*Relative accuracy ratio := Error rates ti*Taq* / *Taq*. A value of 10 indicates 10-fold higher accuracy as compared to *Taq* DNA Polymerase, a value of 1 indicates similar accuracy / precision.

Top quality, recombinant thermostable *Taq* DNA polymerase for automatic "hot start" PCR. Pre-complexed with a specific, thermal dependent PCR inhibitor. Non-antibody mediated, economic "HotStart".

Description:

- tiTaq PCR Master Mix (2x) is a ready-to-use solution containing ti*Taq* DNA Polymerase, optimized reaction buffer, $MgCl_2$ and dNTPs.
- Use of ti*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.
- ti*Taq* DNA Polymerase is a new generation "hot start" enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during normal PCR cycling conditions.
- "Hot start" PCR may increase specificity, sensitivity and yield of a PCR reaction in comparison to the conventional PCR assembly method.
- Thermostable ti*Taq* DNA Polymerase replicates DNA at 72°C and exhibits a half-life of 40 min at 95°C.
- Catalyzes the polymerization of nucleotides into duplex DNA in 5' → 3' direction in the presence of magnesium ions.
- Contains 5' → 3' exonuclease activity.
- Lacks 3' → 5' exonuclease activity ("proofreading").
- Capable of adding extra A to a fraction of 3' ends.
- ti*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 in length.

ti*Taq* PCR Master Mix (2x) contains:

1. ti*Taq* PCR Master Mix (2x)
2. Water, nuclease free
3. 10 x Color Load
4. Thermolabile Uracil N-Glycosylase (UNG)

ti*Taq* PCR Master Mix (2x):

ti*Taq* DNA Polymerase is 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 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.*
2. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I.(1980) Biokhimiya 45, 644.*

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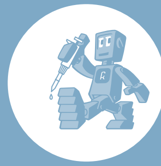

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ti*Taq* Master Mix

"HOT START" PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
ti*Taq* PCR Master Mix (2x)	25 µl	1.25 U ti*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::

$$\text{Template DNA copy number } [\text{molecules}] =$$

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

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

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation Annealing Extension	94-95°C 50-68°C 72°C	15-30 s 30 s 1 min/1 kb	25-35
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. **First Law of PCR:** PCR is comparable to homeopathic processes, working best, if *all* components are added in homeopathic dosages only. Any PCR reaction component, if introduced in excess amounts, beyond the borders of specification, may impair or inhibit the PCR reaction.
2. **Concentration Differences:** Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradient when frozen.
3. **Assembly at Room Temperature**: Prepare reaction mixes at room temperature. Use of ti*Taq* DNA Polymerase allows room temperature reaction setup. Mix well.
4. **Primer Mix.** Primers can be added separately or as a previously prepared primer mix.
5. **Mix Template:** Vortex the samples and briefly spin down.
6. **Cycler Preheating Not Required:** Reactions are placed in a non preheated (room temperature) thermal cycler.
7. **$MgCl_2$:** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in 1x Pol buffers B and C) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, in some cases, reaction may be improved by determining the optimal concentration of $MgCl_2$. dNTP concentrations exceeding 0.2 mM (each) would require additional $MgCl_2$.
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 Mg^{2+}-reaction concentration in 0.5 mM.
Increasing the Mg^{2+} concentration enhances PCR yield but decreases reaction specificity (amplification of more bands, but also of non-specific bands). Decreasing Mg^{2+}-concentration decreases PCR yield but enhances reaction specificity (less bands, but specific PCR products).
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.** Most often, addition of additives / PCR enhancers is not required. 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 10^8 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules). Increased amounts of template DNA (> 0.5 µg) negatively affects / inhibits PCR reactions.

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.** When amplifying long PCR products (> 5 kb):
a. initial denaturation should be 2 min at 94°C
b. cycle denaturation should be 15-20 s at 94°C
c. use an elongation temperature of 68°C instead of 72°C.

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