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TITAQ

tiTaq DNA Polymerase

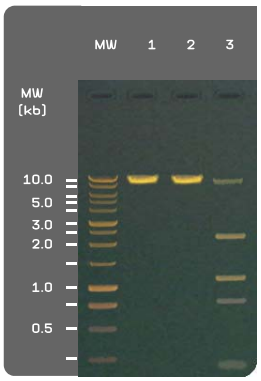
Automatic "Hot Start" PCR system

Taq DNA Polymerase

(Thermus aquaticus)

Cat. No.	Size
E2715-01	200 units
E2715-04	500 units
E2715-02	1000 units
E2715-03	5000 units

Storage Conditions: Store at -20°C



PCR amplification using EURx ti*Taq* DNA Polymerase. A 6.9 kb amplicon of *Bacillus* phage DNA was amplified with ti*Taq* DNA Polymerase. Reactions were incubated at 25°C for 30 min before amplification, allowing for non-specific priming and initial synthesis for unwanted, smaller by-products. Reliable "HotStart" is achieved, if non-specific priming at 25°C is blocked and only one single band is amplified.
Lane M: molecular size marker – Perfect 1 kb plus DNA Ladder (Cat. No. E3131),
Lanes 1,2: PCR amplification reactions using 1.25 U ti*Taq* DNA Polymerase, Pol Buffer B and 0.2 mM dNTPs in 50 µl reaction volume.
Lane 3: PCR amplification reaction using 1.25 U *Taq* DNA Polymerase (plain, non-"HotStart"), Pol Buffer B and 0.2 mM dNTPs in a 50 µl reaction volume.

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:

- 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.
- Use of ti*Taq* DNA Polymerase allows for the increase of PCR specificity, sensitivity and yield in comparison to conventional PCR assembly methods.
- Automatic "hot start" PCR is a fast and convenient method when assembling multiple PCR reactions.
- Both increased specificity and reduced mispriming improve multiplex PCR.
- Eliminated risk of template cross-contamination and assured safe laboratory practice, due to removed necessity to open hot tubes.
- 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.
- Maintains 5' → 3' exonuclease activity.
- Lacks 3' → 5' exonuclease activity ("proofreading").
- Adds extra A at the 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.

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 Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.5% Tween™20, 0.5% Igepal CA-630, 0.1 mM EDTA, 1 mM dithiothreitol, 50 % [v/v] glycerol and stabilizers.

10 x Reaction Buffer:

10 x Pol Buffer A (optimization buffer without $MgCl_2$):
The buffer allows to freely adjust and optimize $MgCl_2$ concentration.

10 x Pol Buffer B (general application, up to 10 kb):
The buffer contains 15 mM $MgCl_2$ and is optimized for use with 0.2 mM of each dNTP.
If required, Mg^{2+}-concentration can be fine tuned to final concentrations exceeding 1.5 mM.

10 x Pol Buffer C (colored):
Similar to 10 x buffer B, but additionally enriched with two gel tracking dyes and a gel loading reagent. The buffer enables direct loading of PCR products to agarose gels.
If required, Mg^{2+}-concentration can be fine tuned to final concentrations exceeding 1.5 mM.

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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"HOT START" PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Pol Buffer A or 10 x Pol Buffer B or 10 x Pol Buffer C	5 µl	1x
25 mM $MgCl_2$	2 - 10 µl when using 10 x Pol Buffer A or 0 – 7 µl when using 10x Pol Buffer B or C	1 – 5 mM 1.5 – 5 mM
dNTP mix (5mM each)	2 µl	0.2 mM of each dNTP
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 µM
ti*Taq* DNA Polymerase, 2.5 U/µl	0.5 µl	1.25 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

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

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

$$\frac{DNA\ amount\ [ng] \cdot 6.022x10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

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

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. **Cycler Preheating Not Required:** Reactions are placed in a non preheated (room temperature) thermal cycler.
5. **$MgCl_2$:** 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).
6. **Colored Loading Buffer:** The 10 x Pol Buffer C allows PCR reactions to be directly loaded onto an agarose gel without prior addition of a gel loading buffer. The buffer contains a gel loading reagent as well as two gel tracking dyes (a red and a yellow dye) that separate during electrophoresis. In a 1 % [w/v] agarose gel the red dye migrates at the same rate as a 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.
7. **Amount of Enzyme:** 1.25 U of ti*Taq* DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme is in excess and will produce satisfactory results. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
8. **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].
9. **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 10^9 molecules, 1 µg of human genomic DNA equals 3 x 10^5 molecules). Increased amounts of template DNA (0.5 µg) may negatively affect or inhibit the PCR reaction.

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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