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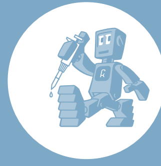

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Tfl DNA Polymerase

(*Thermus flavus*)

Tfl DNA Polymerase
(*Thermus flavus*)

Cat. No.	Size
E1112-01	200 units
E1112-02	1000 units

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmoles of total deoxynucleotides into acid-insoluble material in 30 min at 70°C.

Storage Conditions:
Store at -20˚C

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	20
Amplicon length	to 10 kb
Extension velocity [kb/min]	2 - 4
Generation of 3'-A-overhangs	yes (a fraction of all amplicons)
TA- / blunt cloning possible	yes / yes

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

Thermostable DNA polymerase, suitable for applications requiring high temperature synthesis of DNA.

Description:

- → Efficiently synthesizes DNA at elevated temperatures (1).
- → Used for high temperature DNA sequencing.
- → Broader Mg^{2+} ions concentration tolerance as compared to *Taq* DNA polymerase (3).
- → Enhanced resistance to certain amplification inhibitors present in template DNA isolated from problematic samples (2, 3).
- → Can be used for amplification of nucleic acids in PCR and LCR reaction.

Package Contents

Tfl DNA Polymerase
10x buffer set
 10x Buffer A (no $MgCl_2$)
 10x Buffer B (1.5 mM $MgCl_2$ final)
 10x Buffer C (as buffer B, with two color loading dyes)
$MgCl_2$ solution [25 mM]

Storage Buffer:

50 mM Tris-HCl (pH 7.5 at 22°C), 5 mM dithiothreitol, 0.1 mM EDTA, 50% (v/v) glycerol and stabilizers.

10 x Reaction Buffer:

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

10 x Pol Buffer B (general application):
The buffer contains 15 mM $MgCl_2$ and is optimized for use with 0.2 mM of each dNTP.

10 x Pol Buffer C (coloured):
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.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 90% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. *Kaledin, A. S., Sliusarenko, A.G. and Gorodetskii, S. I. (1980) Biokhimiya 45, 644-651.*
2. *Wiedbrauk D L et al. (1995), J Clin Microbiol. 33 (10): 2643-2646*
3. *Abu Al-Soud, W., Rådström P. (1998) Appl. Environ. Microbiol. (64) 10, 3748-3753*

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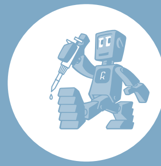

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Tfl DNA Polymerase
PCR PROTOCOL

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 10 x Pol Buffers B or C	1 - 5mM 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
Tfl DNA Polymerase, 5 U/µl	0.25µl	1.25 U (or 1 – 5 U)
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

Notes:

1. **Mix well.** Before use, allow all PCR reaction components including primers and template DNA to thaw completely. Gently vortex and centrifuge all components to avoid localized differences in salt concentration. This is especially important for all magnesium-containing solutions (such as 10x buffers), which are known for their tendency to form concentration gradients during (re-)freezing.
2. **Prepare on ice.** Set up PCR reactions on ice. *Tfl* DNA polymerase exhibits considerable residual activity at room temperature, leading to artifact formation such as primer dimers.
3. **Primer Mix.** Primers can be either added separately or as ready-to-use primer mix prepared separately.
4. **Preheat cycler.** To minimize primer dimer artifact formation, place reactions in a thermal cycler that has been preheated to 94-95°C.
5. **$MgCl_2$ concentration.** Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x Pol Buffers B or 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 optimal concentration of $MgCl_2$. 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.
6. **Color load 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 *Tfl* DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. For certain reactions, enzyme concentration may be adjusted in a range of 1 – 5 U per 50 µl reaction. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
8. **Template copy numbers.** 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).
   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)

Thermal Cycling Conditions:

Step	Temperature	Time	Number of cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation Annealing Extension	94-95°C 50-68°C 72°C	15-60 s 30-60 s 1 min/1 kb	25-35
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. **Annealing temperature.** 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 (exceeding 5 kb in size):
   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.
   An elongation temperature of 68°C or lower is also recommended for the amplification of extremely AT-rich sequence stretches. AT-rich DNA may denature during the 72°C extension step, resulting in an effective stop of the amplification process and PCR failure.

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