Tth DNA Polymerase

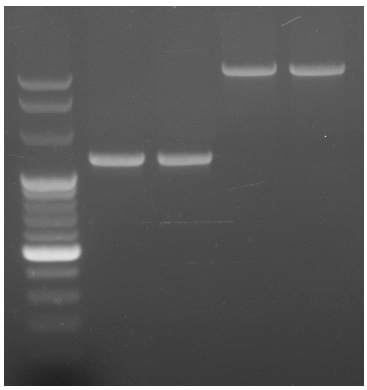
(Thermus thermophilus)

Cat. No.	size
E1115-01	100 units
E1115-02	500 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.

M 1 2 3 4



PCR amplification using EURx Tth DNA Polymerase. A 1.1 kb and 2.5 kb amplicons of the human CCR5 gene were amplified using EURx Tth DNA Polymerase.
Lane M: molecular size marker- Perfect 100 bp DNA Ladder.
Lanes 1, 2: 1.1 kb PCR amplification reactions using 2.5 U Tth DNA Polymerase, Tth PCR Buffer and 0.2 mM dNTPs and 200 ng of human genomic DNA as a template for 35 cycles in 50 µl reaction volume.
Lanes 3, 4: 2.5 kb PCR amplification reactions using 2.5 U Tth DNA Polymerase, Tth PCR Buffer and 0.2 mM dNTPs and 200 ng of human genomic DNA as a template for 35 cycles in 50 µl reaction volume.

Stable thermophilic DNA polymerase, exhibiting reverse transcriptase activity.

Description:

- Tth DNA Polymerase is a thermostable enzyme of approximately 94 kDa from *Thermus thermophilus.*
- Ultrapure, native protein.
- The enzyme replicates DNA at 74°C and exhibits a half-life of 20 min at 95°C
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions and the polymerization of nucleotides into DNA using an RNA template in the 5'→3' direction in the presence of manganese ions.
- Maintains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Suitable for PCR, RT-PCR, reverse transcription and primer extension reactions at elevated temperature (1,2).
- Resistant to amplification inhibitors present in template DNA isolated from problematic samples (3,4).

Storage Buffer:

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

10 x Tth PCR Buffer:

The buffer contains 15 mM $MgCl_2$ and is optimized for use with 0.2 mM of each dNTP.

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. Wang, A. M. , Doyle, M.V. and Mark, D.F. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 9717-9721.

2. Myers, T. W., Gelfanld, D. H. (1991) Biochemistry 30, 7661-6.

3. Kather, H. L., Schwartz, I. (1994) Biotechniques 16, 84-92

4. Poddar, S. K., Sawyer, M. H., Connor, J. D. (1998) J. Med.

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Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Tth PCR Buffer	5 µl	1x
25 mM $MgCl_2$	0-7 µl	1.5-5 mM
dNTP mix (5 mM 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
Tth DNA Polymerase, 5 U/µl	0.5 µl	2.5 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled	Variable	-
Total volume	50 µl	-

Notes:

1. 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.
2. Prepare reaction mixes on ice, mix well.
3. Place reactions in a thermal cycler that has been preheated to 94-95°C.
4. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided with 10 x Tth PCR Buffer) 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 $MgCl_2$.
5. 2.5 U of Tth DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
6. 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%.
7. As a general guide for how much template DNA to use, start with a minimum 104 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 1011 molecules, 1 µg of *E. coli* genomic DNA equals 2 x 108 molecules, 1 µg of human genomic DNA equals 3 x 105 molecules).

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94°C	2-3 min	1
Denaturation	94°C	15-30 s	
Annealing	50-68°C	30 s	25-35
Extension	72°C	1 min/1 kb	
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

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.

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