Perpetual *Taq* DNA Polymerase

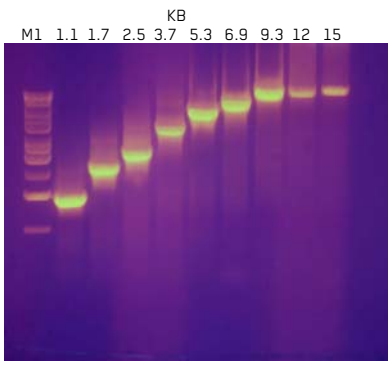
Monoclonal antibody automatic "Hot Start" PCR system

Taq DNA Polymerase
(Thermus aquaticus)

Cat. No.	Size
E2700-01	200 units
E2700-04	500 units
E2700-02	1000 units
E2700-03	5000 units

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



PCR amplification using EURx Perpetual *Taq* DNA Polymerase. Lane M: molecular size marker - Perfect 1 kb DNA Ladder. Lanes 0.5 to 9.3 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U EURx Perpetual *Taq* DNA Polymerase in 50 µl reaction volume.

An initial denaturation step for 3–5 minutes at 95˚C is recommended to ensure a complete denaturation of the antibody,

Pre-complexed with specific anti-*Taq* monoclonal antibody. Top quality thermostable *Taq* DNA polymerase for automatic "hot start" PCR, resulting in greatly enhanced amplification specificity, sensitivity and yield.

Description:

- Ultrapure, recombinant *Taq* DNA Polymerase is reversibly complexed with anti-*Taq* monoclonal antibody that blocks replication activity of the enzyme at moderate temperatures.
- Our carefully selected anti-*Taq* antibodies has high thermal stability, providing protection against non-specific primer extension from room temperature to 70˚C.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94-95˚C for two minutes.
- Formation of complexes between *Taq* DNA Polymerase and an anti-*Taq* antibody forms a basis for automatic "hot start" PCR, which allows for the assembly of PCR reactions at room temperature.
- High stability of the complexes allows for the enormous increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- 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 *Taq* Polymerase replicates DNA at 74˚C and exhibits a half-life of 40 min at 95˚C (1,2).
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.
- Perpetual *Taq* DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products from several hundred bp to 10 kb.

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% glycerol.

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, up to 10 kb):
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 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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Perpetual *Taq* DNA Polymerase
"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 B	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 Buffer 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
Perpetual *Taq* DNA Polymerase, 2.5U/µl	0.5µl	1.25 U
Template DNA	Variable	<0.5 µg/50 µl
Sterile double-distilled water	Variable	-
Total volume	50 µl	-

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	15-60 s 30-60 s 1 min/1 kb	25-35
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. 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 at room temperature. Use of Perpetual *Taq* DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a non preheated (room temperature) thermal cycler.
4. Standard concentration of $MgCl_2$ in PCR reaction is 1.5 mM (as provided in the 1 x Pol Buffer B) 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. 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.
6. 1.25 U of Perpetual *Taq* 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. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
7. 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).

Notes:

1. A 2 min initial denaturation step at 94-95°C is required to inactivate the antibody and restore the polymerase activity.
2. 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.
3. When amplifying long PCR products (over 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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