

Cat. No.: ETAQ-001

ETERNAL[®] LIFE SCIENCE **Taq DNA Polymerase** (ETERBIO-EZ-ETAO-001)

Lot:

Expiry Date: <u>yy</u> / <u>mm</u> / <u>dd</u> Store at -20°C

Origin

E. coli cells that carry the polA gene from Thermus aquatics.

Specification

AppearanceColorless-liquidActivity $\geq 5 \text{ U/}\mu l$

Functions

Deoxynucleoside triphosphate + DNA(n) = diphosphate + DNA(n+1), 5'-3' polymerase and 5'-3' exonuclease, DNA repair, recombination and replication.

Reagents Supplied with Enzyme

10X Eternal Taq Reaction Buffer.

PROPERTIES

Unit Definition One unit is defined as the amount of enzyme that synthesizes 1 nmol of DNA within 3 minutes at 72°C.

Unit Assay Conditions

1X Eternal Taq Reaction Buffer, 125 µM dNTPs and 15 nM primed M13 DNA.

1X Eternal Taq Reaction Buffer

10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween[®] 20, 50% Glycerol.

Molecular Weight Theoretical: 97,000 daltons.

5' - 3' Exonuclease Yes.

3' - 5' Exonuclease No.

Storage

Store at -20 °C. Stable for 1 year in constant freezer temperature. Store at 4 °C for a few weeks. Avoid repeated freeze-thawing cycles.



CERTIFICATE OF ANALYSIS

Endonuclease Assay

No conversion of covalently closed circular DNA to nicked form was detected after incubation of 10 units of *Taq* DNA Polymerase with 1 μ g of supercoiled plasmid DNA (pUC19) in 1X Eternal *Taq* Reaction Buffer for 4 hours at 37 °C.

Exonuclease Assay

No degradation of DNA was observed after incubation of 1 μ g of lambda DNA/*Hin*dIII fragments in 1X Eternal *Taq* Reaction Buffer containing 10 units *Taq* DNA Polymerase for 4 hours at 37 °C. No degradation of DNA was observed after incubation of 1 μ g of lambda DNA/*Hin*dIII fragments in 1X Eternal *Taq* Reaction Buffer containing 10 units *Taq* DNA Polymerase for 4 hours at 37 °C.

Functional Assay

Good performance in PCR was tested for amplification of 1.8 kb gene.

USAGE

We recommend assembling all reaction components on ice and quickly transferring the reaction to a thermocycler preheated to the denaturation temperature $(95^{\circ}C)$.

Component	50 µl reaction	Final Concentration
10X <i>Taq</i> Reaction Buffer	5 µl	1X
Primer A	Variable	0.1–1.0 μΜ
Primer B	Variable	0.1–1.0 μΜ
10 mM dNTPs	1 µl	$200\mu M$ of each dNTP
Template DNA	Variable	< 1.0 µg
Taq DNA Polymerase	0.5 µl	2.5 U/50 µl PCR
Nuclease-free water	to 50 µl	

Thermocycling conditions for a routine PCR.

Step	Temperature	Time
Initial Denaturation	95°C	30 seconds
30 Cycles	95℃ 45-68℃ 72℃	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	72°C	5 minutes
Hold	4-10°C	

*Mg²⁺ and additives

The optimal Mg^{2+} concentration of 1.5 mM empirically, as provided in the 1X Eternal *Taq* Reaction Buffer, will generate satisfactory amplification of most amplicons. However, in some difficult targets, Mg^{2+} can be improved by increasing 0.5-1.5 mM. Amplification of some cases, reactions may be improved with additives, like DMSO.

For Research Use Only

Rev. 1