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AMPLIBIOTHERM DNA POLYMERASE

DESCRIPTION:

Amplibiotherm DNA Polymerase is a thermostable 94 kDa DNA Polymerase purified from E.coli PVG-AI recombinant strain expressing ***Thermus aquatiqus*** polymerase gene.
The enzyme catalyzes polymerisation of nucleotides into duplex DNA in the 5'-3' direction in presence of Mg++ ions. The enzyme possesses also a 5'-3' exonuclease activity. Amplification of target **DNA fragments <100 b.p. up to 10.000 b.p.** can be achieved with this enzyme.

CONCENTRATION:

5 units/ul

Description	FS-T-002
Amplibiotherm Taq DNA Polymerase	250 U
10X Reaction Buffer	1 vial
25mM MgCl2 separately	1 vial

UNIT DEFINITION:

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

STORAGE AND DILUTION BUFFER:

20 mM Tris-HCl , 1 mM DTT; 0.1 mM EDTA;
100 mM NaCl , Stabilizer ; 50% glycerol pH: 7.5(25°C)
buffer is optimized to use with 0.2mM for each dNTPs

STORAGE TEMPERATURE:

Store Amplibiotherm DNA Polymerase below 0°C, preferably at -20° C, in a constant temperature freezer.

EXPIRY DATE:

1 year upon receipt.

10X REACTION BUFFER:

100mM Tris-HCl, 500mM KCl, pH 9.0 (25°C).

REACTION BUFFER	
10X Reaction Buffer (contains 15mM $MgCl_2$; included)	Cat. No. **FS-B-006**
10X Reaction Buffer (without $MgCl_2$; plus 25 mM $MgCl_2$ separately)	Cat. No. **FS-B-007**

Protocol for routine *Taq* PCR reaction.

On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

For 50 ul PCR Reaction	Volume	Final Conc.
Amplibiotherm DNA Polymerase (5U/ul)	0.25 ul	1.25 U
10X PCR Buffer	5 ul	1 X
dNTP mix (2.5 mM each)	4 ul	200 uM each
Template	< 500 ng	< 500 ng
Forward Primer	5 ~ 50 pmol	0.1~1 uM
Reverse Primer	5 ~ 50 pmol	0.1~1 uM
Distilled water	up to 50 ul	

Gently mix the reaction and spin down in microcentrifuge.
If the termocycler does not have a heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation.

Cycling conditions for a routine PCR reaction:

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	25 ~40
Anneal	50~65	10 ~ 30 sec.	
Extend	72	10 ~ 60 sec.	
Final Extension	72	5 min.	1

Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

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Quality Control Analysis:

Unit Characterization Assay

Specific activity was measured using a 2-fold serial dilution method.Dilutions of enzyme were made in 1X reaction buffer. Reactions were incubated 10 minutes at 75°C, plunged on ice, and analyzed using the method of Sambrook and Russell.

Protein Concentration (OD280) Measurement

A 2.0 µl sample of enzyme was analyzed at OD280 using a spectrophotometer standardized using a 2.0 mg/ml BSA sample and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 110,380 and molecular weight of 93,909 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 µl of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µl of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample,confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µl reaction containing 11,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µl of enzyme solution incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 µl reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µl of enzyme solution incubated for 4 hours at 37ºC resulted in less than 0.5% release of TCA-soluble counts.

Endonuclease Activity

A 50 µl reaction containing 0.5 µg of pBR322 DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

E.coli 16S rDNA Contamination Test

Replicate 5 µl samples of enzyme solution were denatured and screened in a TaqMan qPCR assay for the presence of contaminating E.coli genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

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