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Product Information Sheet *P7250L Rev E*

Product Information	
Taq-B DNA Polymerase	
Part Number	P7250L 280375
Concentration	5,000 U/mL
Unit Size	10,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	74,625 U/mg
SS Exonuclease	50 U <5.0% released
DS Exonuclease	50 U <1.0% released
DS Endonuclease	50 U = No conversion
***E.coli* DNA Contamination**	50 U <10 copies

Product Description:

Taq-B DNA Polymerase is a thermally stable, processive, 5′→3′ DNA polymerase. The 94 kDa protein possesses an inherent 5′→3′ nick-translation moiety and lacks a 3′→5′ proofreading function.

Source of Protein

A recombinant *E. coli* strain carrying the Taq DNA polymerase gene from the thermophilic organism *Thermus Aquaticus* YT-1.

Supplied in

20 mM Tris-HCl
100 mM NaCl
1.0 mM DTT
0.1mM EDTA
Stabilizer
50% glycerol
pH 7.5 @ 25°C

Supplied with

B7030 (10X PCR Buffer I)

10X PCR Buffer I (B7030):
100 mM Tris-HCl
500 mM KCl
15 mM $MgCl_2$
pH 8.3 @ 25°C

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

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 and added to 50 µL reactions containing Calf Thymus DNA, 25 mM TAPS (pH 9.3), 50 mM KCl, 2.0mM $MgCl_2$, 1 mM DTT, ^{3}H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 75°C, plunged on ice, and analyzed using the method of Sambrook and Russell (*Molecular Cloning, v3, 2001, pp. A8.25-A8.26*)

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) 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 93,910 Daltons.

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.

Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µl reaction containing 10,000 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.

(additional information on reverse side)

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 1.0% release of TCA-soluble counts.

Double-Stranded 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 5uL 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 (C_t) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control C_t values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

Notes:

Taq DNA Polymerase is the original and most commonly used PCR enzyme. Taq excels at amplifying shorter (<5 kb) sequences from low-complexity template sources and produces robust yields with little or no optimization of reaction conditions. Consider the following guidelines when designing PCR strategies using Taq DNA Polymerase.

1. **DNA Template:** Although extensive purification of PCR templates is typically not necessary, care should be taken with crude or partially purified DNA sources as handling and chemical agents can adversely affect the PCR process. Exposure to short-wave UV light or other DNA damaging agents should be avoided, as should high ionic strength, detergents such as SDS, loading dyes and phenol. In order to prevent contamination from previous PCR reactions, consider setting up reactions in a positive-pressure hood and with aerosol barrier pipet tips. In a typical 25 cycle PCR, 10^4 copies of target sequence will yield reproducible amplification product. This corresponds to roughly 0.1-1 ng/ml (final concentration) of plasmid DNA, and 1-10 µg/ml of genomic DNA. The use of lower DNA concentrations typically produces less non-specific product, while higher concentrations can allow for fewer cycles and lower mutation rates.
2. **Primer Design:** Ideally, oligonucleotide primers are 15-30 bases in length, nearly 50% G+C, and have equal (+/- 3°C) annealing temperatures. The use of software to detect self-complementary or hairpin-prone regions is advised and is offered as a service by some synthesis providers. Note that although the 5'-terminus of the primer may contain untemplated sequence, the 3' end must match perfectly. Typical oligonucleotide concentration in the reaction is 0.1-0.5 µM.
3. **Magnesium:** Magnesium is a critical component of the PCR reaction though its concentration can be modulated to promote various effects. Generally, 1.5-2.0 mM Mg2+ is targeted, but higher concentrations (up to 5 mM) may be used to stimulate the yield of reactions at the expense of fidelity. The converse is also true – lower magnesium concentrations will promote higher- fidelity products with a lower overall amplification yield. Note that certain reaction components, in particular template DNA and oligonucleotides, may contribute chelating agents to the reaction which could lower the effective magnesium concentration and starve the reaction.
4. **dNTPs:** Generally, a final concentration of 100-200 µM dNTPs is employed, though higher concentrations may stimulate yields (particularly with longer targets) and lower may offer increases in fidelity. Taq DNA Polymerase can also incorporate and read through deoxy Uridine and Inosine, two analogs used in certain applications.
5. **Taq Polymerase:** 1 unit/50 µL reaction (20 U/mL) is typical, though additional enzyme may be added to stimulate yields. Taq DNA Polymerase extends a DNA template at approximately 2000 nucleotides/minute, so it is recommended that 45-60 seconds of extension time be provided per cycle. Appropriate extension temperatures range from 66-72°C.

Usage Instructions:

Typical 50 µL Reaction

On ice, prepare each of following master mixes, combine, and place in heated (to 94°C) thermal cycler:

2X DNA/Oligonucleotide Master Mix:

1.0 µL 10 mM dNTPs
1.0 µL 10 µM Forward Primer
1.0 µL 10 µM Reverse Primer
1.0 µL 500 ng/µL genomic DNA
21 µL Type I Water

2X Enzyme/Buffer Master Mix:

5.0 µL 10X PCR Buffer I
0.2 µL 5 U/µL Taq DNA Polymerase
19.8 µL Type I Water

General Cycling Conditions

94°C	3 minutes	Initial Denaturation
25 Cycles:		
94°C	30 seconds	Denaturation
55°C	30 seconds	Annealing
68°C	30 seconds	500 bp extension
68°C	5 minutes	Final Extension

Legal Disclaimers:

Patents

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