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Product Information Sheet *G5020L Rev.B*

Product Information	
Thermolabile UDG	
Part Number	G5020L 280465
Concentration	1,000 U/mL
Unit Size	500 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST	**SPECIFICATION**
Purity (SDS-PAGE)	>98%
Specific Activity	30,000 U/mg
SS Exonuclease	10 Units < 1.0% release
DS Exonuclease	10 Units < 1.0% release
DS Endonuclease	5 Units = No conversion
***E.coli* DNA Contamination**	5 Units < 10 Copies

Product Description:

Thermolabile Uracil-DNA Glycosylase removes uracil from DNA by hydrolyzing the N-glycosylic bond between the deoxyribose and the base leaving an AP (apurinic or apyrimidinic) site. This enzyme (1-10 units) is completely inactivated by a 10 minute incubation at temperatures greater than 50°C in the 1x reaction buffer as measured in the unit characterization assay.

Source of Protein

A recombinant *E. coli* strain carrying the recombinant thermolabile Uracil DNA Glycosylase gene isolated from a marine bacteria.

Supplied in

50 mM Tris-HCl
50 mM NaCl
1 mM DTT
0.1 mM EDTA
50% glycerol
pH 7.5 @ 25°C

Supplied with

B5020 10X Reaction Buffer

10X Reaction Buffer (B5020)

700 mM Tris-HCl
100 mM NaCl
10 mM EDTA
1 mg/mL BSA
pH 8.0 @ 25°C

Unit Definition

1 unit is defined as the amount of Thermolabile UDG required to release 1nmol of Uracil from dU-containing DNA in one hour at 37°C.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer (70mM Tris-HCl, 10mM NaCl, 1mM EDTA, 100 µg/mL BSA, pH 8.0 @ 25°C) and added to reactions containing a ^{3}H-dUTP labeled 1.1kb PCR product in 1X reaction buffer. Reactions were incubated for 60 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-2000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209), and blanked with product storage buffer. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 41,820 and molecular weight of 26,218 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 same enzyme species. 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 98% 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 1.0% 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 37°C 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 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 (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:

Thermolability was examined by heat treating 10 Units of enzyme for 10 minutes at 50°C in 1X reaction buffer. The enzyme was transferred to reactions containing a ^{3}H-dUTP 1.1kb PCR product and activity was measured as described in the unit characterization assay. After heat treatment, less than 0.5% activity remains.

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Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.