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Product Information Sheet G5010L Rev F

Product Information	
Uracil DNA Glycosylase (UDG)	
Part Number	G5010L 280455
Concentration	2,000 U/mL
Unit Size	10,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST	**SPECIFICATION**
Purity (SDS-PAGE)	>99%
Specific Activity	77,000 U/mg
SS Exonuclease	100 U < 5.0% released
DS Exonuclease	100 U < 1.0% released
DS Endonuclease	100 U = no conversion
***E.coli* DNA Contamination**	100 U < 10 copies

Product Description:

Uracil-DNA Glycosylase catalyzes the hydrolysis of the N-glycosylic bond between the uracil and sugar, leaving an abasic site in uracil-containing single or double-stranded DNA. The enzyme shows no measurable activity on short oligonucleotides (<6 bases), or RNA substrates.

Source of Protein

A recombinant *E. coli* strain carrying the Uracil DNA Glycosylase gene from *E. coli* K-12.

Supplied in

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

Supplied with

B5010 (10X UDG Reaction Buffer)

10X UDG Reaction Buffer (B5010):
200mM Tris-HCl
10 mM DTT
10 mM EDTA
pH 8.0@ 25°C

Unit Definition

1 unit is defined as the amount of enzyme that catalyzes the release of 1.8 nmol of Uracil in 30 minutes from double-stranded, tritiated, Uracil containing-DNA at 37°C in 1X UDG Reaction Buffer.

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 a ^{3}H-dUTP PCR product and 1X UDG Reaction Buffer. Reactions were incubated for 10 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-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 40,540 and molecular weight of 25,693 Daltons.

SDS-Page (Physical Purity Assessment)

2.0 µL of 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 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.

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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.