

Product Information	
Thermolabile UDG	
Part Number	G5020L
Concentration	1,000 U/mL
Unit Size	500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

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 (1,2). This enzyme (1-10 units) is completely inactivated by a 10 minutes incubation at temperatures greater than 50°C in the 1x reaction buffer as measured in the unit characterization assay.

Product Specifications						
G5020						
Assay	SDS Purity	Specific Activity	SS Exonuclease	DS Exonuclease	DS Endonuclease	<i>E. coli</i> DNA Contamination
Units Tested	n/a	n/a	10	10	5	5
Specification	>98%	30,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying the recombinant thermolabile Uracil DNA Glycosylase gene isolated from a marine bacterium.

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

Molecular weight: 26.2 KDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer (70 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA, 100 µg/mL BSA, pH 8.0 at 25°C) and added to reactions containing a ³H-dUTP labeled 1.1 kb 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 (OD₂₈₀) is determined by OD₂₈₀ absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution 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.

Supplied in:

50 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Supplied with:

10X Reaction Buffer (B5020): 700 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, 1 mg/mL BSA (pH 8.0 at 25°C)

Usage Instructions:

1. Use the included UDG reaction buffer (B5020) at 1X. This enzyme is active in most molecular biology reaction buffers, so there is no need to exchange buffers.
2. Add 0.5 µL UDG (G5020) to a reaction with uracil-containing DNA substrate (up to 0.1 µg) in 20 µL total reaction.
3. Incubate at 25-37°C for 30 minutes.
4. This enzyme can be completely inactivated by a 10 minutes incubation at temperatures greater than 50°C.

Note: protocol can be modified for application specific usage.

References:

1. Lindahl, T. et al. (1977) J. Biol. Chem., 252, 10, 3286-3294.
2. Longo, M.C. et al. (1990) Gene, 93, 125-128.

Disclaimer:

Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.

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. SDS sheets relevant to this product are available upon request.