



Human Uracil DNA N-Glycosylase (UNG)

Catalog Number: EN001

Specifications and Use

Contents	Component	Size
	Human Uracil DNA N-Glycosylase	200 Units in 0.2 mL
	10X DDR Buffer 1 (200 mM Tris-Cl (pH 8.0), 10 mM EDTA, 10 mM DTT, 1 mg/mL BSA)	1 mL
Description	<ul style="list-style-type: none">Human UNG is one of four known human DNA N-glycosylases involved in the removal of uracil from DNA.¹ Human UNG is a monofunctional enzyme that initiates base excision repair (BER) by hydrolyzing the N-glycosidic bond between the uracil base and the sugar-phosphate backbone on the DNA. The resultant abasic or AP site generated by this cleavage is repaired in subsequent steps of the BER process.²The presence of uracil in DNA can occur by spontaneous deamination of cytosine or by misincorporation of dUMP (instead of dTMP) during DNA replication.² Failure to repair the uracil base in U:G mismatches (that arise from cytosine deamination) prior to replication will result in a G:C to A:T transition mutation. U:A mismatches (that occur from replicative error) while not miscoding, may be converted to cytotoxic and mutagenic AP sites during the DNA repair process.	
Source	<ul style="list-style-type: none">A cDNA corresponding to amino acid residues 84 - 304 of human UNG³ including the uracil DNA N-glycosylase catalytic domain⁴ was fused to a sequence encoding a 10X histidine epitope tag at the carboxy-terminus. The recombinant fusion protein was expressed in <i>E. coli</i>.	
Unit Definition	<ul style="list-style-type: none">One unit is the amount of enzyme required to cleave the uracil-containing oligonucleotide duplex indicated below at the rate of 1 pmole/hour at 37° C.Sequence: 5' C C T G C C C T G <u>U</u> G C A G C T G T G G G 3' 3' G G A C G G G A C A C G T C G A C A C C C 5'	
Specificity	<ul style="list-style-type: none">Human UNG hydrolyzes uracil from single stranded or double stranded DNA. In addition to uracil, it also recognizes 5-fluorouracil, 5-hydroxyuracil, isodialuric acid, and alloxan.	
Assay Conditions	<ul style="list-style-type: none">Serial dilutions of human UNG are incubated for 1 hour at 37° C in a 20 µL reaction containing 1X DDR Buffer 1 and 4 pmole of Uracil Oligonucleotide (Catalog # 3852-100-OL) labeled with ³²P and annealed with 4 pmole Oligo Complement B (Catalog # 3852-100-OL). Following the addition of 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, 0.2% bromophenol blue), samples are heated to 95° C for 10 minutes and fast cooled on ice. Cleavage products are resolved by 20% polyacrylamide gel electrophoresis, excised from the gel, and quantified by scintillation counting.	
Storage Buffer	<ul style="list-style-type: none">20 mM HEPES (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA, and 50% (v/v) glycerol.	
Storage	<ul style="list-style-type: none">Product is stable for at least 6 months from the date of receipt if stored at ≤ -20° C in a manual defrost freezer.For long term storage, aliquot the enzyme and buffer and store at ≤ -80° C.Avoid repeated freeze-thaw cycles.	
References	<ol style="list-style-type: none">Sharer, O.D. and J. Jiricny (2001) <i>BioEssays</i> 23:270.Friedberg, E.C. <i>et al.</i> (1995) in <i>DNA Repair and Mutagenesis</i>, ASM Press, Washington, DC.Olsen, L.C. <i>et al.</i> (1989) <i>EMBO J.</i> 8(10):3121.Slupphaug, G. <i>et al.</i> (1995) <i>Biochemistry</i> 34:128.	

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

R&D Systems, Inc.
1-800-343-7475