

E. coli* Uracil-N-Glycosylase (UNGase)*Catalog #:** 4025-100-EB**Contents:** Uracil-N-Glycosylase
10X REC™ Buffer 6**Size:** 100 units
1 mL

Description: Uracil bases in DNA form by deamination of cytosine, giving rise to C/G to T/A transitions. A known mechanism to correct this DNA base mutation in *E. coli* utilizes Uracil-N-Glycosylase, a DNA glycosylase that removes uracil to generate an AP site.

Source: Purified from *E. coli* containing a recombinant plasmid harboring the *E. coli ung* gene.

Unit Definition: One unit is the amount of enzyme required to cleave a uracil-containing oligonucleotide within an oligonucleotide duplex at the rate of 1 pmole/hour at 37° C.

Specificity: Uracil-N-Glycosylase hydrolyzes uracil from single-stranded or double-stranded DNA (see enzyme activity synopsis on reverse), but not from oligonucleotides with 6 or fewer bases. It also recognizes 5-fluorouracil, 5-hydroxy-uracil and isodialuric acid.

Assay Conditions: 1X REC Buffer 6 (20 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA), 4 pmole Uracil Oligonucleotide (Cat.# 3852-100-OL) labeled with ³²P, 4 pmole Oligo Complement B (Cat.# 3852-100-OL), and serial dilutions of Uracil-N-Glycosylase in a 20 µL reaction volume are incubated for 1 hour at 37° C. For analysis, 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, 0.2% bromophenol blue) are added, the samples are heated to 95° C for 10 minutes then fast cooled to 2 - 8° C, and cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are cut out and radioactivity counted to quantify cleavage products.

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA, and 50% (v/v) glycerol.

Storage Conditions: Store at -20° C in a manual defrost freezer. For long term storage, aliquot and store at -80° C. Avoid repeated freeze-thaw cycles.

References: 1. Duncan, B.K., (1981) DNA glycosylases in *The Enzymes* (Boyer, P.D., ed), pp. 565-586. New York: Academic Press.
2. Friedberg, E.C., et al. (1995) *DNA Repair and Mutagenesis*. American Society of Microbiology, Washington D.C.:ASM Press.
3. Verri, A., et al. (1992) *Uracil-DNA glycosylases preferentially excise mispaired uracil*. *Biochemistry Journal* **287**:1007.

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