Product Information	
10X Uracil Cleavage System	
Part Number	Y9180L
Unit Size	750 Reactions
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: The Uracil Cleavage System provides two enzymes, which, when added sequentially to a reaction containing a synthetic DNA fragment containing a deoxy-uracil, generate a single nucleotide gap at the location of the uracil residue. The system consists of two individual enzyme components, Uracil DNA Glycosylase (UDG) and Endonuclease VIII, provided at a 10X concentration to be added to a reaction containing a uracil-containing polynucleotide sequence. UDG catalyzes the excision of the uracil base, creating an abasic site with an intact phosphodiester backbone (1,2). The lyase activity of Endonuclease VIII breaks the phosphodiester backbone at both 3' and 5' to the abasic site, liberating the deoxyribose sugar (3,4).

Product Specifications					
Y9180					
Assay	**SDS Purity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**10µL**	**10µL**	**10µL**	**5µL**
Specification	>99%	<5.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: Each component protein is purified separately from *E. coli* strains containing recombinant Endonuclease VIII or Uracil-DNA Glycosylase gene.

Unit Definition. UDG (G5010): 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.

Unit Definition. Endonuclease VIII (Y9080): 1 unit is defined as the amount of enzyme required to cleave 1 pmol of an oligonucleotide duplex containing a single AP site in 1 hour at 37°C.

Quality Control Analysis:

UDG Unit Activity is 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 3H-dUTP containing 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.

Endonuclease VIII Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 10 µL reactions containing 2.0 µM of a FAM-labeled, 34-base duplex oligonucleotide, containing a single Uracil. [Note: substrate pre-treated for 2 minutes with UDG to create an abasic site] Reactions were incubated 60 minutes at 37°C, plunged on ice, denatured with N-N-dimethyl -formamide and analyzed on a 15% TBE-Urea acrylamide gel.

Contamination Tests (performed on UDG and Endonuclease VIII separately):

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 **r**eplicate 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 with UDG (G5010): 10mM Tris-HCl, 50 mM NaCl, 1mM DTT, 0.1mM EDTA, 50% glycerol (pH 7.5 at 25°C)
Supplied with Endonuclease VIII (Y9080): 10mM Tris-HCl, 250 mM NaCl, 0.1mM EDTA, 50% glycerol (pH 8.0 at 25°C)

Usage Instructions:
The volume can be adjusted to suit the requirement of the application by maintaining the ratios of components outlined below for 10 µL reaction.

1. Prepare Uracil-containing DNA (e.g., PCR amplification product). The Uracil Cleavage System enzymes are active in most molecular biology reaction buffers, so there is no need to exchange buffers prior to assembling the cleavage reaction.
2. Add 0.5 µL UDG (G5010) to a reaction vessel containing the DNA substrate (final concentration up to 300nM) in 1X reaction buffer.
3. Add 0.5 µL Endonuclease VIII (Y9080) to the above.
4. Incubate at 37°C for 30 minutes.
5. Following the incubation period, the reaction can be stopped by adding a gel-loading stop solution in preparation for electrophoretic analysis or prepared for transformation. In the case of transformation, the fragment may be ligated into a cloning vector using T4 DNA Ligase (L6030-HC).

References:
1. Lindhal, T. et al. (1977) J. Biol. Chem., 252, 3286-3294.
2. Lindhal, T. (1982) Annu. Rev. Biochem., 51, 61-64.
3. Melamede, R.J. et al. (1994) Biochemistry, 33, 1255-1264.
4. Jiang, D. et al. (1997) J. Biol. Chem., 272, 32230-32239.

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