

Product Information	
10X Uracil Cleavage System	
Part Number:	Y918L
Lot Number (G501):	
Lot Number (Y908):	

Certificate of Analysis

2010-Y918-Rev.C

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 catalyses 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 both 3' and 5' to the abasic site, liberating the deoxyribose sugar (3,4).

Source of Protein:

Each component protein is purified separately from *E. coli* strains containing recombinant Endonuclease VIII and Uracil-DNA Glycosylase genes.

Supplied in:

Each enzyme component provided in this buffer:

12.5 mM Tris-HCl
33 mM KCl
33 mM NaCl
1 mM dithiothreitol
0.3 mM EDTA
50% glycerol
pH 7.7 @ 25°C

Unit Definition, UDG

1 unit is defined as the amount of enzyme that catalyzes the release of 60 nmol of uracil in 30 minutes from double-stranded, tritiated, uracil containing-DNA at 37°C in 1X UDG Reaction Buffer.

(additional information on reverse side)

Product Specification*	
Unit Size:	750 Reactions
Volume:	0.375 ml each component
Purity (SDS-PAGE)	>99%
SS Exonuclease	10 µL <5.0% released
DS Exonuclease	10 µL <0.5% released
Endonuclease	10 µL <10% converted
<i>E. coli</i> 16S rDNA Contamination	10 µL <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below.

Unit Definition, Endo VIII

One 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:

Unit Characterization Assay, UDG

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer ([UDG]_f = 0.825-0.006µg/µL) and added to 50 µL reactions containing 15,000 CPM of a ³H-dUTP containing 1.1kb 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.

Unit Characterization Assay, Endo VIII

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer ([EndoVIII]_f = 0.02-0.008µg/µL) and added to 10 µL reactions containing 2.0 µM of an FAM-labeled, 34-base, duplex oligonucleotide, containing a single Uracil. [Note: substrate pre-treated for 2 minutes with 1 unit of UDG to create an a basic site] Reactions were incubated 15 minutes at 37°C, plunged on ice, denatured with N-N-dimethylformamide and analyzed on a 15% TBE-Urea acrylamide gel.

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.

Nuclease Contamination Tests, Performed on

UDG and Endo VIII separately:

Single-Stranded Exonuclease Activity

A 50 μL reaction containing 11,000 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.

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 0.5% release of TCA-soluble counts.

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.

Protocol

The following reaction specification is set to 10 μL . The volume can be tailored up or down to suit the requirement of the application by maintaining the ratios of components outlined below.

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. Combine and mix the following components in a sterile tube:

Amount	Description	Final Concentration
1-10 μL	Uracil-containing DNA (up to 3 pmol)	Up to 300 nM
to 10 μL	Type I Water	N/A
10 μL	Total Volume	

3. Add 0.5 μL UDG (G501) to a reaction vessel containing the DNA substrate.
4. Add 0.5 μL Endo VIII (Y908) to a reaction vessel containing the DNA substrate.
5. Incubate at 37°C for 30 minutes.
6. 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 (Part Number: L603-HC-L).

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.



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