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Product Information

10X Uracil Cleavage System

Catalog Number **KEM0011**
Storage Temperature –20 °C
Unit Size 750 Reactions

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.

Reagents

Uracil DNA Glycosylase (UDG)
Catalog Number KEM0001A
10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT,
0.1 mM EDTA, 50% glycerol, pH 7.5

Endonuclease VII
Catalog Number KEM0007A
10 mM Tris-HCl, 250 mM NaCl, 0.1 mM EDTA,
50% glycerol, pH 8.0

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Unit Definition UDG
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
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.

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:

Component	Volume (µL)	Final Concentration
Uracil-containing DNA (up to 3pmol)	1-10 µL	Up to 300 nM
Sterile Water	X µL	N/A
Total Volume	**10 µL**	

3. Add 0.5 µL UDG (KEM0001A) to a reaction vessel containing the DNA substrate.
4. Add 0.5 µL Endonuclease VII (KEM0007A) 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, Catalog Number KEM0019.

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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