IFU0108 Rev 0 Status: RELEASED printed 12/8/2016 1:48:51 PM by Trevigen Document Control

Related Products:

DNA Repair Enzymes:

Catalog #	Description	Size
4150-010-EB	*Sulfolobus silfataricus* DNA Polymerase IV (Dpo4)	10 µg
4045-01K-EB	*E. coli* Endonuclease III	1000 U
4050-100-EB	*E. coli* Endonuclease IV	100 U
4065-100-EB	Chorella Virus Pyrimidine Dimer Glycosylase	1000 U
4130-100-EB	Human 8-oxo-G DNA Glycosylase (hOGG1)	100 U
4040-100-EB	*E. coli* Fpg	500 U
4025-100-EB	*E. coli* Uracil-N-Glycosylase	100 U
4000-500-EB	*E. coli* MutY DNA Glycosylase	500 U
4110-01K-EB	Human AP Endonuclease	1000 U
4020-01K-EB	Human β Polymerase	1000 U
4120-100-EB	Human FEN-1	100 U
4125-100-EB	*E. coli* Mismatch Uracil DNA Glycosylase (Mug)	100 U
4090-100-EB	Mouse 3-Methyladenine DNA Glycosylase (Aag)	100 U
4070-500-EB	Thermostable thymine mismatch DNA Glycosylase	500 U
4055-100-EB	T4 Endonuclease V	100,000 U

Antibodies:

Catalog #	Description	Size
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg
4350-MC-100	Anti-UVssDNA (clone C3B6)	100 µg
4354-MC-050	Anti-8-oxo-dG (clone 2E2)	50 µl
4360-MC-100	Anti-BPDE (clone 8E11)	100 µg
4411-PC-100	Anti-γ-H2AX polyclonal	100 µl
4410-PC-100	Anti-FEN-1 polyclonal	100 µl

DNA damage characterization:

Catalog #	Description	Size
4100-100-FK	*S. pombe* UVDE FLARE Kit	100 µl

Accessories:

Catalog #	Description	Size
9804-050-P	TreviGel 500	50 g
9850-250-6	Orange G Loading Buffer	6 x 250 µl

***S. pombe* UVDE**
Catalog #:4100-100-EB
Lot # : 21434J10
Storage: -80 °C
TREVIGEN®
1-800-873-8443

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

S. pombe UVDE

Catalog #: 4100-100-EB

Contents:		**Size:**
4100-100-01	*S. pombe* UVDE	100 µl
3900-500-05	10X REC™ Buffer 5	500 µl

Description: Exposure to UV irradiation can damage DNA through the formation of bipyrimidine DNA photoproducts that are potentially cytotoxic and mutagenic. The two major classes of dimeric photoproducts are cyclobutane pyrimidine dimers and (6-4)-photoproducts (see figure 1).

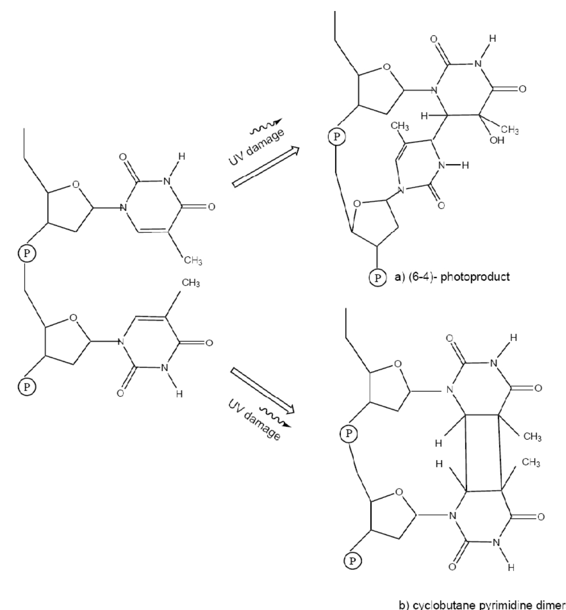

Figure 1. Photoproducts recognized by UVDE. Ultraviolet radiation leads to the formation of a variety of photoproducts involving pyrimidines. The most common are cyclobutane pyrimidine dimers that can be repaired by photolyases. (6-4)-photoproducts are rarer and significantly distort the DNA helix.

Ultraviolet Damage Endonuclease (UVDE) is a nuclease involved in the repair of bipyrimidine DNA photoproducts and acts as an alternative to base or nucleotide excision repair pathways. Repair of pyrimidine dimers by UVDE-mediated excision occurs by cleavage immediately 5' to the photoproduct site on the DNA strand in an ATP-independent manner, and is more efficient *in vitro* than nucleotide excision repair.

Source: Trevigen's UVDE is a fusion protein consisting of glutathione-S-transferase fused to a truncated form of the UVDE protein from *Schizosaccharomyces pombe* (GST-Δ228-UVDE). Unlike full length UVDE, which has proved to be unstable and difficult to solubilize, GST-Δ228-UVDE lacks 228 amino acids from the amino terminus (of UVDE) and is soluble and stable when stored at -80°C. The presence of the N-terminal deletion does not affect the native properties of the protein, and optimal activity occurs at 30°C.

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Substrate Specificity: Enzymatic studies revealed that UVDE from *Schizosaccharomyces pombe* recognizes pyrimidine dimers, 6-4 photoproducts, apurinic/apyrimidinic sites, uracil, dihydrouracil, and other non-UV-induced DNA adducts.[1,2] Biochemical and genetic analysis also suggest that UVDE may be involved in orchestrating mismatch repair *in vivo*,[3] and this enzyme is also active on insertion-deletion loops.[4]

Storage Buffer: 50 mM Tris-Cl (pH 6.0), 10 mM glutathione, and 10% glycerol.

Storage Conditions: Store at -80 ºC. For long term storage, freeze in working aliquots at -80 ºC to avoid repeated freeze-thawing.

Assay Conditions & Analysis: UVDE treatment of supercoiled DNA, containing adducts such as cyclobutane pyrimidine dimers, leads to relaxation of the supercoiling resulting in a relative shift in electrophoretic mobility. This may be visualized in agarose gels by staining with an intercalating dye such as ethidium bromide. Qualitative evaluation is performed by comparing the relative amounts of DNA contained within bands corresponding to supercoiled DNA (ccc) and to nicked (relaxed) forms. There are other more sensitive dyes available (e.g. SYBR® Gold, (Molecular Probes)), which reduce the amount of DNA required per lane. The actual concentrations of DNA and enzyme, and incubation times may have to be adjusted for optimal results. For increased sensitivity, or if a specific gene or DNA strand is under investigation, the DNA may be transferred by Southern blot to a membrane and probed with radiolabeled DNA fragments from the DNA strand or gene of interest.

Materials and Reagents:

- 30ºC Incubator
- Agarose Gel Electrophoresis Equipment and Reagents
- UV transilluminator
- Test and Control DNA
- Pipettors and tips
- Ice Bath
- 10 mg/ml EtBr in water

Method:

1. Prepare the test DNA at approximately 250 ng/µl in water. In assays utilizing other forms of DNA, e.g. mitochondrial DNA, the concentration of DNA may need to be increased. Avoid exposure of the DNA to UV and solar light during preparation and storage. Always run controls to check for damage that may be introduced during DNA preparation and handling.
2. For a 20 µl reaction mix combine the following on ice in microcentrifuge tubes:

Component	Tube 1	Tube 2	Tube 3	Tube 4
Test plasmid DNA (250 ng/µl)	1	1	-	-
Control plasmid DNA (250 ng/µl)	-	-	1	1
10X REC™ Buffer 5 (cat# 3900-500-05)	2	2	2	2
dH_2O	16	17	16	17
GST-Δ228-UVDE (cat# 4100-100-01)	1	-	1	-

3. Incubate all tubes at 30°C for 15 to 60 minutes.
4. Add 2 µl of 10X EDTA Loading Buffer (see appendix) to each tube.
5. Resolve DNA by agarose gel electrophoresis (e.g. use 1.0% Trevigel 5000 (cat# 9806-050-P)).
6. Stain gel for 15 minutes in 0.5 µg/ml ethidium bromide in water.
7. Visualize by viewing under ultraviolet light.

Interpretation of results: By agarose gel electrophoresis, supercoiled plasmid DNA migrates faster than either nicked or linear DNA. The different migration patterns of the DNA forms and relative DNA content in each band allows for interpretation of conversion from supercoiled to nicked and/or linear DNA. Figure 2 illustrates relaxation of supercoiling by GST-Δ228-UVDE on plasmid DNA exposed to UV.

A 3.2 kb purified plasmid was treated with or without 1000 J/m2 UV at 254 nm. Approximately 1 µg of DNA was treated with or without 250 ng of GST-Δ228-UVDE for 45 minutes at 30°C and analyzed by electrophoresis on a 1 % Trevigel 5000 gel in 1X TAE buffer. The gel was stained with 0.5 µg/ml ethidium bromide for 15 minutes then photographed under ultraviolet light. GST-Δ228-UVDE does not exhibit activity on unmodified DNA (lane 2) and UV treatment alone does not alter the ratio of the DNA forms of the plasmid (lane 3). Treatment of UV irradiated DNA with GST-Δ228-UVDE converted the covalently closed circular plasmid to nicked and linear forms.

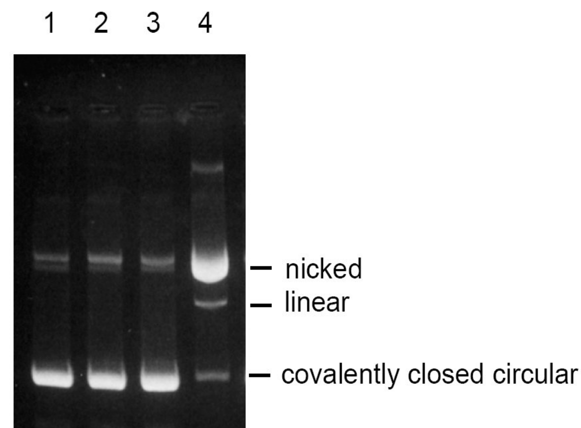

Figure 2: UVDE treatment of uv-irridiated plasmid DNA: Lanes 1, 2, untreated plasmid DNA; lanes 3 and 4, plasmid irradiated with 100 J/m^2 UV; Lanes 2, and 4, DNA treated with 250 ng GST-Δ228-UVDE.

Note: When smaller quantities of DNA are treated with UVDE, some nicking activity may occur in control DNA reflecting UV damage in plasmid preparation.

Buffer Composition:

1. **10X REC™ Buffer 5**: 200 mM HEPES pH 6.5; 100 mM $MgCl_2$; 10 mM $MnCl_2$; 1M NaCl
2. **10X EDTA Loading Buffer**: 25% Ficoll; 2% SDS; 10 mM EDTA pH 8.0
3. **0.5 µg/ml ethidium bromide**: Prepare a 10 mg/ml stock solution of ethidium bromide in water. Store in a dark or foil covered bottle at room temperature. Dilute 10 mg/ml stock solution 1 in 2000 in water, sufficient for gel immersion. The diluted stock may be reused several times if stored in the dark. Dispose of according to state and federal regulations.

References:

1. Avery AM, Kaur B, Taylor JS, Mello JA, Essigmann JM, Doetsch PW (1999) Substrate specificity of ultraviolet DNA endonuclease (UVDE/Uve1p) from *Schizosaccharomyces pombe*. Nucleic Acids Res 27: 2256–64.

2. Kanno S, Iwai S, Takao M, Yasui A (1999) Repair of apurinic/ apyrimidinic sites by UV damage endonuclease; a repair protein for UV and oxidative damage. Nucleic Acids Res 27: 3096–103.

3. Kaur B, Fraser JLA. Freyer GA, Davey S, Doetsch PW (1999) A Uve1p-mediated mismatch repair pathway in *Schizosaccharomyces pombe*. Mol Cell Biol 19:4703–10.

4. Doetsch, P.W., Beljanski, V., and Song, B. (2006). The ultraviolet damage endonuclease (UVDE) protein and alternative excision repair: a highly diverse system for damage recognition and processing. In DNA Damage Recognition, V. Beljanski and B. Song, eds. (New York: Taylor & Francis Press), pp. 211–223.