S. pombe UVDE
Catalog #: 4100-100-EB
Lot #: 21434J10
Storage: -80 °C

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).

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.

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.
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. Biochemical and genetic analysis also suggest that UVDE may be involved in orchestrating mismatch repair in vivo, and this enzyme is also active on insertion-deletion loops.

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

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

Materials and Reagents:
- 30°C 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test plasmid DNA (250 ng/μl)</td>
<td>1</td>
</tr>
<tr>
<td>Control plasmid DNA (250 ng/μl)</td>
<td>1</td>
</tr>
<tr>
<td>10X REC™ Buffer 5 (cat# 3900-500-05)</td>
<td>1</td>
</tr>
<tr>
<td>dH2O</td>
<td>1</td>
</tr>
<tr>
<td>GST-Δ228-UVDE (cat# 4100-100-01)</td>
<td>1</td>
</tr>
</tbody>
</table>

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 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).

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-irradiated plasmid DNA: Lanes 1, 2, 3, and 4, DNA treated with 250 ng GST-Δ228-UVDE.](image)

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

References: