Endonuclease V

Description: Endonuclease V is a repair enzyme found in E. coli that recognizes deoxyinosine, a deamination product of deoxyadenosine in DNA. Endonuclease V, often called deoxyinosine 3’-endonuclease, recognizes DNA containing deoxyinosines (paired or not) on double-stranded DNA, single-stranded DNA with deoxyinosines and to a lesser degree, DNA containing abasic sites (ap) or urea, base mismatches, insertion/deletion mismatches, hairpin or unpaired loops, flaps and pseudo-Y structures. It is believed that Endonuclease V needs another protein to repair the DNA, as it does not remove the deoxyinosine or the damaged bases (1,2,3).

Endonuclease V cleaves the second phosphodiester bond 3’ to the mismatch of deoxyinosine (2), leaving a nick with 3’-hydroxyl and 5’-phosphate (4).

Source: An E. coli strain containing a gene fusion found in the Endo V gene and the gene coding for the maltose binding protein (MBP). The fusion protein is purified to near homogeneity and is active as a fusion. The protein contains 223 amino acids and has a molecular weight of 24.9 kDa (5).

Applications:
- Mismatch Cleavage
- Cleavage of oligonucleotides containing deoxyinosine and a weaker affinity for oligonucleotides containing base mismatches (5)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
pH 7.9 @ 25°C

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single deoxyinosine site in a total reaction volume of 10 µl in 1 hour at 37°C.

*A deoxyinosine site is synthetically prepared with a dI in the middle of one strand of a 34 mer oligonucleotide duplex.

Diluent Compatibility: Diluent Buffer C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Unit Assay Conditions: 1X NEBuffer 4 containing 10 pmol of fluorescently labeled oligonucleotide duplex in a total volume of 10 µl.

Quality Control Assays
Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Endonuclease V with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA (10^5 cpm/µg) in NEBuffer 1 for 4 hours at 37°C released < 0.1% of the total radioactivity.

(see other side)
Enzyme Properties

Activity in NEBuffers:

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEBuffer 1</td>
<td>75%</td>
</tr>
<tr>
<td>NEBuffer 2</td>
<td>50%</td>
</tr>
<tr>
<td>NEBuffer 3</td>
<td>100%</td>
</tr>
<tr>
<td>NEBuffer 4</td>
<td>100%</td>
</tr>
</tbody>
</table>

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Heat Inactivation: 65°C for 20 minutes.

References: