**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 ura, 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 and third phosphodiester bonds 3’ to the mismatch of deoxyinosine with a 95% efficiency for the second bond and a 5% efficiency for the third bond (2), leaving a nick with 3´-hydroxyl and 5´-phosphate (4).

**Source:** An *E. coli* strain containing a gene fusion of 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, 10 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 1X NEBuffer 4.

**Reaction Conditions:**
- 10X 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 dl 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 (105 cpm/µg) in NEBuffer 1 for 4 hours at 37°C released < 0.1% of the total radioactivity.

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 75%
- NEBuffer 2: 50%
- NEBuffer 3: 100%
- NEBuffer 4: 100%

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:**

**Figure 1:** 5’ fluorescently labeled oligonucleotides contain a 6-carbon phosphodiester spacer that have a structure that may be similar to a urea moiety. Removal of the 5’ fluorescent label from an oligonucleotide by Endo V has been observed (6).