Endonuclease VIII

While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has β and δ lyase activity while Endonuclease III has β lyase activity.

Source: An E. coli strain which carries the cloned neI gene

Applications:
- Single cell gel electrophoresis (Comet assay) (3,4,5)
- Alkaline elution (6)
- Alkaline unwinding (7)

Reagents Supplied with Enzyme:
10X Endonuclease VIII Reaction Buffer.

Reaction Conditions: 1X Endonuclease VIII Reaction Buffer. Incubate at 37°C.

1X Endonuclease VIII Reaction Buffer:

- 10 mM Tris-HCl
- 75 mM NaCl
- 1 mM EDTA
- pH 8.0 @ 25°C

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 AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.
* An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Assay Conditions: 1X Endonuclease VIII Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Recommended Dilution for Comet Assay:
1:10⁴ to 1:10⁵ (3,4,5,8). A detailed protocol can be found at www.neb.com.

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

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 25 units of Endonuclease VIII for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Endonuclease VIII with 1 µg of a mixture of single and double-stranded [³²P] E. coli DNA (10⁵ cpm/µg) for 4 hours at 37°C released < 0.4% of the total radioactivity.

Heat Inactivation: 250 units of enzyme were inactivated by incubation at 75°C for 10 minutes.

Usage Note: Endonuclease VIII will remove deoxyribose- 5’ phosphate dR5’P at a nicked site (9).

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References: