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.

Unit Assay Conditions: 1X NEBuffer 3 containing 10 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Quality Assurance: Purified free of contaminating exonucleases and endonucleases.

Recommended Dilution for the Comet Assay: 1.10^4 to 1:10^5 (2,3,4,7). A detailed protocol can be found at www.neb.com.

Heat Inactivation: 85°C for 20 minutes.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the reagents for stability.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 100 units of Endonuclease IV incubated 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 IV with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 100 units of Endonuclease IV with 1 µg of X174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

References:

7. Marks, K., New England Biolabs, Inc. unpublished observations.

Figure 1: The 6-carbon phosphodiester spacer used in the 3’ fluorescent label of synthetic oligonucleotides is structurally similar to an abasic site. Removal of the fluorescent label from the 3’ end of oligonucleotide substrates by Endo IV has been observed (8).