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

Unit Definition: One unit is defined as the amount of enzyme required to cleave 20 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 20 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 4 containing 20 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Recommended Dilution for the Comet Assay: 1:10³. A detailed protocol can be found at www.neb.com

Heat Inactivation: 65°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 enzyme for stability.

SS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [³²P] denatured DNA (2 x 10⁶ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.30% radioactivity.

DS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [³²P] DNA (2 x 10⁶ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.23% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg φX174 RF I DNA for 4 hours at 37°C in 50 µl 1X Buffer 4 resulted in < 10% conversion to RF II.

References:

Figure 1: The 6-carbon phospodiester 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 APE 1 has been observed (11).
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