Ethenoadenosine Oligonucleotide and Complement D

**Catalog #:** 3863-100-OL

**Contents:**
- Ethenoadenosine Oligonucleotide
- Oligo Complement D

**Size:**
- 100 pmol
- 100 pmol

**Description:** This set consists of an oligonucleotide containing a single ethenoadenosine base and a complementary oligonucleotide, serving as an excellent substrate for 3-Methyladenine DNA Glycosylase (Aag) (Cat.# 4090-100-EB). Aag removes etheno adenosine leaving an abasic site that may be detected by cleavage with *E. coli* Endonuclease IV (Cat.# 4050-100-EB), Human AP Endonuclease (Cat.# 4110-100-EB), or by alkali cleavage with 3X Alkali Loading Buffer (see below). The oligonucleotides may be conveniently labeled with γ-32P-ATP and polynucleotide kinase using the OligoTAG™ Kinase Kit (Cat.# 9500-25-K).

**Sequence:**
5′ CCTGCCCTGAGCA aGCTGTGGG 3′
3′ GGACGGGACTCG TCGACACCC 5′

**Assay Conditions:** 1X REC™ Buffer 9 (10 mM HEPES-KOH, pH 7.4, 100 mM KCl, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT), 1 pmole Ethenoadenosine Oligonucleotide labeled with 3P, 1 pmole Oligo Complement D, and serial dilutions of enzyme in a reaction volume of 20 µL are incubated for 1 hour at 37° C. For analysis, 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, and 0.2% bromophenol blue) is added, the samples are heated to 95° C for 10 minutes and then fast cooled to 2 - 8° C. The cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are cut out and the radioactivity is counted to quantify the cleavage products.

**Storage Conditions:** Store at -20° C in a manual defrost freezer. For long term storage, aliquot and store at -80° C. Avoid repeated freeze-thaw cycles.

**References:**
1. Dosanjh, M.K. et al. (1994) 1,N6-ethenoadenosine is preferred over 3-methyladenine as substrate by a cloned human N-methylpurine-DNA glycosylase (3-methyladenine-DNA glycosylase). Biochemistry 33:1624.

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