

# hAAG



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M0313S 002150117011

## M0313S



**500 units**    **10,000 U/ml**    **Lot: 0021501**

**RECOMBINANT** Store at **-20°C** Exp: **1/17**

**Description:** Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N<sup>6</sup>-ethenoadenine and hypoxanthine. hAAG catalyzes the hydrolysis of the N-glycosidic bond to release the damaged base. hAAG is also known as methylpurine DNA glycosylase (MPG) (1,2,3) or 3-methyladenine-DNA glycosylase (ANPG) (4).

**Source:** An *E. coli* strain which carries the cloned truncated human AAG gene (1)

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### Applications:

- Single cell gel electrophoresis (Comet Assay) (5,6,7)
- Alkaline elution (8)
- Alkaline unwinding (9)

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween 20, 0.5% NP-40 and 50% glycerol.

### Reagents Supplied with Enzyme:

10X ThermoPol Buffer.

### Reaction Conditions:

1X ThermoPol Buffer. Incubate at 37°C.

### 1X ThermoPol Reaction Buffer:

10 mM KCl  
10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
20 mM Tris-HCl  
2 mM MgSO<sub>4</sub>  
0.1% Triton X-100  
pH 8.8 @ 25°C

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

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**Unit Definition:** One unit is defined as the amount of enzyme required to create an AP site from 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.

**Unit Assay Conditions:** 1X ThermoPol Buffer containing 5 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

**Molecular Weight:** 25,752 Daltons

### 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 100 units of hAAG incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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**Exonuclease Activity:** Incubation of a 50 µl reaction containing 50 units of hAAG in NEBuffer 1 with 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA (10<sup>5</sup> 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 hAAG with 1 µg φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFLI as determined by agarose gel electrophoresis.

**Heat Inactivation:** 65°C for 20 minutes.

(see other side)

CERTIFICATE OF ANALYSIS

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**References:**

1. O'Brien, P. and Ellenberger, T. (2003) *Biochemistry* 42, 12418–12429.
2. Abner, C.W. et al. (2001) *J. Biol. Chem.* 276, 13379–13387.
3. Samson, L. (1991) *Proc. Natl. Acad. Sci.* 88, 9127–9131.
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6. Collins, A. et al. (1993) *Carcinogenesis* 14, 1733–1735.
7. Collins, A. et al. (1996) *Environmental Health Perspectives* 104, 465–469.
8. Pflaum, M. et al. (1998) *Free Rad. Res.* 29, 585–594.
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