hAAG

1-800-632-7799
info@neb.com
www.neb.com

M0313S 002120714071

M0313S

500 units **10,000 U/ml** **Lot: 0021207**

RECOMBINANT **Store at –20°C** **Exp: 7/14**

Description: Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N^6-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)

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 $(NH_4)_2SO_4$
20 mM Tris-HCl
2 mM $MgSO_4$
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.

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.

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 [^{3}H] *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 hAAG with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Heat Inactivation: 65°C for 20 minutes.

(see other side)

CERTIFICATE OF ANALYSIS

hAAG

1-800-632-7799
info@neb.com
www.neb.com

M0313S 002120714071

M0313S

500 units 10,000 U/ml Lot: 0021207

RECOMBINANT Store at –20°C Exp: 7/14

Description: Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N6-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)

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 (NH4)2SO4
20 mM Tris-HCl
2 mM MgSO4
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.

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.

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 [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 hAAG with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Heat Inactivation: 65°C for 20 minutes.

(see other side)

CERTIFICATE OF ANALYSIS

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.
4. Lau, A.Y. et al. (1998) *Cell*, 95, 249–258.
5. Singh, N. et al. (1961) *Experimental Cell Reseach* 175, 184–191.
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.
9. Hartwig, A. et al. (1996) *Toxicology Letters* 88, 85–90.

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.
4. Lau, A.Y. et al. (1998) *Cell*, 95, 249–258.
5. Singh, N. et al. (1961) *Experimental Cell Reseach* 175, 184–191.
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.
9. Hartwig, A. et al. (1996) *Toxicology Letters* 88, 85–90.

Page 2 (M0313)