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AM03160PU-S Monoclonal Antibody to 8-Hydroxy-Guanosine / 8-OHG - Purified

Alternate names: 8-Hydroxy-2'-deoxyguanosine, 8-Hydroxyguanine, 8-Hydroxyguanosine, 8-OFdG, 8-OHG, 8OG, 8OHG, 8OHdG

Quantity: 25 µg

Concentration: 1.0 mg/ml

Background: DNA or RNA damage is due to environmental factors and normal metabolic processes inside the cell, that then hinder the ability of the cell to carry out its functions. There are four main types of DNA due to endogenous cellular processes and they are oxidation, alkylation, hydrolysis and mismatch of the bases. During the oxidation of bases, highly reactive chemical entities collectively known as RONS, occurs. RONS stands for reactive oxygen and nitrogen species and includes nitric oxide, superoxide, hydroxyl radical, hydrogen peroxide and peroxynitrite. Numerous studies have shown that RONS causes a variety of issues including DNA damage (1). 8-hydroxyguanine, 8-hydroxy-2'-deoxyguanonsine and 8-hydroxyguanosine are all RNA and DNA markers of oxidative damage. 8-hydroxy-2'-guanosine is produced by reactive oxygen and nitrogen species including hydroxyl radical and peroxynitrite. Specifically its high biological relevance is due to its ability to induce G to T transversions, which is one of the most frequent somatic mutations (2). 8-hydroxy-guanine has been the most frequently studied type of DNA base damage, with studies in diabetes, and cancer. Base modifications of this type arise from radical-induced hydroxylation and cleavage reactions of the purine ring (3, 4). And finally, 8-hydroxy-guanosine, like 8-hydroxy-2'-guanosine, induces a mutagenic transversion of G to T in DNA. Its role has specifically been tested in the development of diabetes, hypertension and strokes (5, 6, and 7).

Host / Isotype: Mouse / IgG2b

Recommended Isotype Controls: SM12P, AM03110PU-N

Clone: 15A3

Immunogen: 8-Hydroxy-Guanosine-BSA and –Casein conjugates

Format:
State: Liquid purified Ig fraction
Purification: Affinity Chromatography on Protein G
Buffer System: PBS containing 50% Glycerol and 0.09% Sodium Azide

Applications:
ELISA.
Dot Blot.
Immunoaffinity Chromatography.
Immunohistochemistry (1/1000).
Immunocytochemistry.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Recognizes markers of Oxidative Damage to DNA (8-Hydroxy-2'-deoxyguanosine, 8-Hydroxyguanine and 8-Hydroxyguanosine).

Storage: Store undiluted at 2-8°C up to one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Product Citations:

Originator or purchased from resellers:

1. Zawada WM, Mrak RE, Biedermann J, Palmer QD, Gentleman SM, Aboud O, et al. Loss of angiotensin II receptor expression in dopamine neurons in Parkinson's disease correlates with pathological progression and is accompanied by increases in Nox4- and 8-OH guanosine-related nucleic acid oxidation and caspase-3 activation. Acta Neuropathol Commun. 2015 Feb 3;3:9. doi: 10.1186/s40478-015-0189-z. PubMed PMID: 25645462.
2. Aboud O, Mrak RE, Boop FA, Griffin WS. Epilepsy: neuroinflammation, neurodegeneration, and APOE genotype. Acta Neuropathol Commun. 2013 Jul 29;1:41. doi: 10.1186/2051-5960-1-41. PubMed PMID: 24252240.
3. Brennan LJ, Haukedal JA, Earle JC, Keddie B, Harris HL. Disruption of redox homeostasis leads to oxidative DNA damage in spermatocytes of Wolbachia-infected Drosophila simulans. Insect Mol Biol. 2012 Oct;21(5):510-20. doi: 10.1111/j.1365-2583.2012.01155.x. Epub 2012 Jul 26. PubMed PMID: 22831171.
4. Angeloni C, Malaguti M, Rizzo B, Barbalace MC, Fabbri D, Hrelia S. Neuroprotective effect of sulforaphane against methylglyoxal cytotoxicity. Chem Res Toxicol. 2015 Jun 15;28(6):1234-45. doi: 10.1021/acs.chemrestox.5b00067. Epub 2015 May 11. PubMed PMID: 25933243.
5. Advancing age increases sperm chromatin damage and impairs fertility in peroxiredoxin 6 null mice. Redox Biology. [Epub ahead of print]. 2015. Ozkosem, B., Feinstein, S.I., Fisher, A.B. and O'Flaherty, C.
6. Zawada WM, Mrak RE, Biedermann J, Palmer QD, Gentleman SM, Aboud O, et al. Loss of angiotensin II receptor expression in dopamine neurons in Parkinson's disease correlates with pathological progression and is accompanied by increases in Nox4- and 8-OH guanosine-related nucleic acid oxidation and caspase-3 activation. Acta Neuropathol Commun. 2015 Feb 3;3:9. doi: 10.1186/s40478-015-0189-z. PubMed PMID: 25645462.

General Readings:

1. Kim H.W., Murakami A., Williams M.V., and Ohigashi H. (2003) Carcinogenesis 24(2): 235-241.
2. Pilger A. and Rudiger H.W. (2006) Int Arch Occup Environ Health. 80(1): 1-15.
3. Malins D.C. and Haimanot R. (1991) Cancer Res. 51(19): 5430-5432.
4. Kvam E. and Tyrrell R.M. (1997) Carcinogenesis 18(11): 2281-2283.
5. Kowluru R.A., Atasi L., and Ho Y.S. (2006) Invest Ophthalmol Vis Sci 47(4): 1594-9.
6. Bowers R. et al. (2004) Am J Respir Crit Care Med. 169(6): 764-9.
7. Cui J., Holmes E.H., Greene T.G., and Liu P.K. (2000) Faseb J. 14(7): 955-67.

Protocols:

Immunostaining with 8-OHdG Monoclonal Antibody AM03160PU (Clone 15A3)

Tissue Preparation

8-OHdG monoclonal antibody reacts on both 50 um frozen tissue sections and paraffin-embedded sections.
Tissue should be dissected fresh and fixed in periodate-lysineparaformaldehyde

(PLP) at 4°C overnight.
PLP
Heat 1 L dH_2O to 60°C.
Add 60 g paraformaldehyde.
Add 33 g dibasic $NaPO_4$.
Cool to RT in a cold water bath.
Add 9 g monobasic $NaPO_4$.
Add 6.45 g Na-*m*-periodate.
Add 41.1 g lysine (HCl salt).
Filter and dilute to 3 L with dH_2O.
Adjust pH to 7.6 with 1.0 N NaOH approx. (20-30 ml).

Tissue prepared for frozen sectioning must be cryoprotected in a 20% glycerol-2% DMSO solution in phosphate buffer for 24-48 hours. Tissue will sink to the bottom of container when fully penetrated. This will eliminate freezing artifact from cutting.

Glycerol-DMSO (for 3 L)
2.4 L 0.1M phosphate buffer
600 ml glycerol
60 ml DMSO

0.1 M Phosphate Buffer, pH 7.4 (for 1 L)
1 L dH_2O
11 g dibasic $NaPO_4$
3 g monobasic $NaPO_4$
After frozen sectioning, tissue should be stored in phosphate buffer with 0.08% sodium azide.

Staining Sections By DAB Procedure
Paraffin-embedded sections must be deparaffinized by sequential immersion in the following for 3 minutes each: xylene (twice), absolute ethanol (twice). Agitate gently in each solution. Proceed with the following procedure.
1. Pretreat sections with a methanolperoxide solution to eliminate endogenous peroxidases.
Methanol-Peroxide
100 ml absolute methanol
1 ml 33% H_2O_2
Incubate sections in methanolperoxide solution for 30 minutes at RT.

2. Wash sections 3 times for 10 minutes each in 0.1 M phosphate buffered saline (PBS)
PBS, pH 7.4 (for 1 L)
1 L dH_2O
11 g dibasic $NaPO_4$
3 g monobasic $NaPO_4$
8.5 g NaCl

3. Incubate sections for 1 hour in 10% normal goat serum in PBS.
4. Incubate sections in the primary antibody for 18-24 hours at RT. Depending on the nature of the sample, a shorter incubation time may be used.
It is recommended that a concentration range of 1-10 ug/ml be evaluated in order to determine the optimal concentration for each type of tissue sample. Dilute antibody in PBS containing 0.3% Triton X-100, 0.08% sodium azide and 2% normal goat serum.
NOTE: A humidified chamber is necessary when staining paraffin sections. Slides should be placed flat and primary antibody applied over the section, covering it completely.
5. Rinse sections 3 times for 10 minutes each in PBS.
6. Incubate for 3 hours with peroxidase-conjugated goat antimouse IgG (Boehringer-Mannheim, Indianapolis, IN) diluted 1:300 in PBS with 2% normal goat serum.
7. Rinse sections 3 times for 10 minutes each in PBS.
8. Incubate sections for 5-10 minutes in a solution of 0.5 mg/ml 3,3' diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO) and 0.005% hydrogen peroxide in 0.05 M tris HCl buffer, pH 7.6 plus imidazole (10 ml/110 ml Tris buffer).

50 mM Tris Buffer, pH 7.6
1 L dH_2O
6 g Trizma base
3 ml concentrated HCl (37%)

Sodium Imidazole
100 ml 0.1 M phosphate buffer
0.7 g sodium imidazole

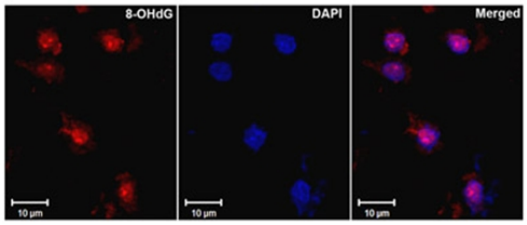
9. Rinse sections 3 times for 10 minutes each in PBS.
10. Mount free-floating sections on subbed slides and air dry.

Subbing Solution
500 ml dH_2O
2.5 g gelatin
0.25 g chromium potassium sulfate
Heat to 60°C. Filter and proceed to coat slides. Once slides are air dried, sections can be mounted.

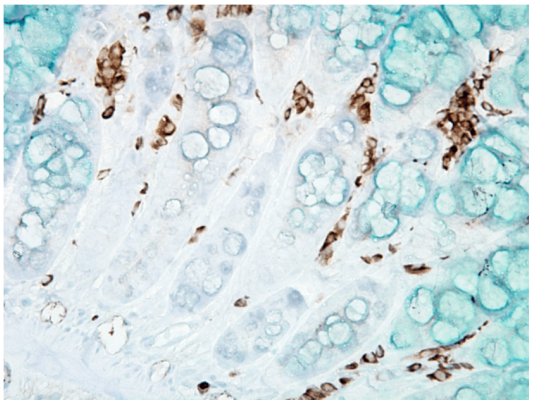
11. Dehydrate mounted/paraffin sections by sequential immersion in the following for 3 minutes each: 70% ethanol, 95% ethanol, absolute ethanol, xylene. Agitate gently in each solution.
12. Apply coverslip with Permount in a chemical fume hood.

Pictures:

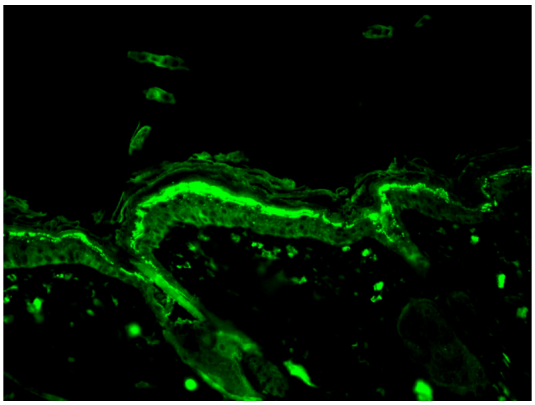
AM03160PU used against oxidized 8-OH-dG in ischemic Rat brain tissue (Left Panel). Center Panel: DAPI staining. Right Panel: merged. *Courtesy of Dr. Yang, University of New Mexico, USA.*



Immunohistochemistry analysis using Mouse Anti-DNA Damage Monoclonal Antibody, Clone 15A3 (AM03160PU). Tissue: inflamed colon. Species: Mouse. Fixation: Formalin. Primary Antibody: Mouse Anti-DNA Damage Monoclonal Antibody (AM03160PU) at 1:1000000 for 12 hours at 4°C. Secondary Antibody: Biotin Goat Anti-Mouse at 1:2000 for 1 hour at RT. Counterstain: Mayer Hematoxylin (purple/blue) nuclear stain at 200 µl for 2 minutes at RT. Magnification: 40x.



Immunohistochemistry analysis using Mouse Anti-DNA Damage Monoclonal Antibody, Clone 15A3 (AM03160PU). Tissue: backskin. Species: Mouse. Fixation: Bouin's Fixative and paraffin-embedded. Primary Antibody: Mouse Anti-DNA Damage Monoclonal Antibody (AM03160PU) at 1:100 for 1 hour at RT. Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:50 for 1 hour at RT.



DNA Damage visualized on a retinal injury model using AM03160PU. *Courtesy of Dr. Rajashekhar Gangaraju, University of Indiana, Department of Opthamology, Eugene and Marilyn Glick Eye Institute.*