Anti-DNA/RNA Damage
Catalog# SMC-155 C/D

Size: 25/100µg

This product is for *in vitro* research use only and is not intended for use in humans or animals

Product	**Mouse monoclonal DNA/RNA oxidative damage Antibody**
Clone	**15A3**
Immunogen	**8-hydroxy-guanosine-BSA and –casein conjugates**
Host and Subclass	**Mouse, IgG_{2B}**
Cited Applications	**ELISA, IH, may also be used on immunoaffinity columns**
Specificity	**Recognizes markers of oxidative damage to DNA (8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanine and 8-hydroxyguanosine)**
Species cross-reactivity	**N/A**
Format	**Protein G-purified antibody in PBS, in 50% glycerol, 0.1% sodium azide.**
Concentration and working dilution	**0.65mg/ml; See assay instructions below.**
Storage and stability	**-20˚C; 1 year+; shipped on cold packs or ambient**

Scientific 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).

Selected References

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.

Immunostaining with 8-OHdG Monoclonal Antibody (SMC-155)

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-lysine-paraformaldehyde (PLP) at 4^{o}C vernight.

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PLP

Heat 1 L dH_2O to 60^{o}C.

Add 60 g paraformaldehyde.
Add 33 g dibasic $NaPO_4$.

Cool to room termperature 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 0.1 M 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 methanol-peroxide solution to eliminate endogenous peroxidases.

Methanol-Peroxide
100 ml absolute methanol
1 ml 33% H_2O_2

Incubate sections in methanol-peroxide solution for 30 minutes, room temperature.

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 room temperature. 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 anti-mouse 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' diamino-benzidine 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^{o}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

Material Safety Data Sheet

Anti-DNA Damage (Monoclonal Antibody) SMC-155

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The below information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. StressMarq shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalogue for additional terms and conditions of sale.

Hazardous Ingredients

The physical, chemical and toxicological properties of these components have not been fully investigated. It is recommended that all laboratory personnel follow standard laboratory safety procedures when handling this product. Safety procedures should include wearing OSHA approved safety glasses, gloves and protective clothing. Direct physical contact with this product should be avoided.

Known Hazardous Components	CAS Number	Percent
Sodium Azide	26628-22-8	0.1

Physical Data

This product consists of mouse immunoglobulin in PBS in 50% glycerol shipped on gel packs. The physical properties of this product have not been investigated thoroughly.

Fire and Explosion Hazard and Reactivity Data

NOT APPLICABLE

Toxicological Properties

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

Spill and Leak Procedures

Observe all federal, state and local environmental regulations.

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Dispose or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.