bio-techne® | R&D SYSTEMS

Catalog Number 4354-MC-050

Volume 50 µL

8-oxo-dG Antibody

Product Description

This mouse monoclonal antibody specifically binds to 8-hydroxy-2′-deoxyguanosine within DNA in H_2O_2-treated cells. It is used to detect oxidative damage by ELISA and immunocytochemistry. Sufficient antibody is provided for approximately 50 slides, when a 1:250 dilution is used.

Physical State

This antibody is provided as purified immunoglobulin from mouse ascites at 0.5 mg/mL in 1X PBS containing 0.1% sodium azide and 50% glycerol.

Clone

15A3

Ig Class

IgG_{2B}

Storage Conditions

Store the unopened product at -20 to -70 °C. Use a manual defrost freezer and avoid repeated freeze-thaw cycles. Do not use past expiration date.

Applications

Immunodetection of 8-oxo-dG by ELISA, immunocytochemistry, and immuno-fluorescence. Empirical determination will be required for optimal results. For optimal outcomes, cells should be grown on a surface that allows for fixation and direct labeling such as sterile chamber slides and coverslips. Alternatively, paraffin-embedded samples may be used.

Immunocytochemistry Protocol

1. Plate cells 5 x 10^4 cells (sub-confluent) on cover slips or chamber slides o/n.
2. Aspirate medium, wash cells with 1X PBS, and treat with 300 µL of 100-300 µM H_2O_2 in 1X PBS on ice for 20 minutes. (Be sure to establish untreated controls).
3. Wash 3X with 1X PBS and fix with -20 °C MeOH followed by -20 °C acetone at -20 °C for 15 minutes each. Alternatively, cells may be fixed with 1:1 MeOH acetone for 20 minutes at -20 °C. Allow to air dry.
4. Treat fixed cells with 0.05 N HCl for 5 minutes on ice.
5. Wash 3X with 1X PBS for 5 minutes each.
6. Incubate with 250 µL of 100 µg/mL RNAse in 150 mM NaCl and 15 mM sodium citrate for 1 hour at 37 °C.
7. Wash sequentially in 1X PBS, 35%, 50% and 75% EtOH for 3 minutes each.
8. Denature DNA in situ with 250 µL 0.15 N NaOH in 70% EtOH for 4 minutes.
9. Wash briefly 2X with 1X PBS.
10. Use 0.2 µg/mL (250 µL) Hoechst 33342 in 1X PBS to stain DNA for 10 minutes.
11. Wash sequentially in 70% EtOH containing 4% v/v formaldehyde, 50% and 35%EtOH, and 1X PBS for 2 minutes each.
12. Incubate in 250 µL of 5 µg/mL Proteinase K in 20 mM Tris, 1 mM EDTA, and pH 7.5 (TE) for 10 minutes at 37 °C.
13. Wash several times with 1X PBS.

Immunocytochemistry Protocol

14. Block non-specific binding with 5% normal goat serum in 1X PBS for 1 hour at room temperature.

15. Wash 3X with 1X PBS and incubate with 250 µL anti-8-hydroxyguanine antibody at a concentration of 1:250 diluted in 1X PBS containing 1% BSA, 0.01% Tween 20 at 4 °C o/n in a humidified chamber.

16. Wash several times with 1X PBS containing 0.05% Tween 20 for 5 minutes each.

17. Incubate cells in 250 µL of fluorescent secondary antibody conjugate and goat anti-mouse IgG (Alexa Fluor 488 (Molecular Probes)) at 5 µg/mL in 1X PBS containing 1% BSA for 1 hour **in the dark** at room temperature.

18. Wash several times with 1X PBS containing 0.05% Tween 20.

19. Rinse with deionized water.

20. Mount with appropriate mounting media.

Note: The number of cells (adherent/suspended) to be plated and, concentrations of primary, and secondary antibodies have to be optimized/titrated by the end user. Include appropriate controls such as, a) omission of primary antibody; b) omission of secondary antibody.

Example Results

FIGURE // 1A

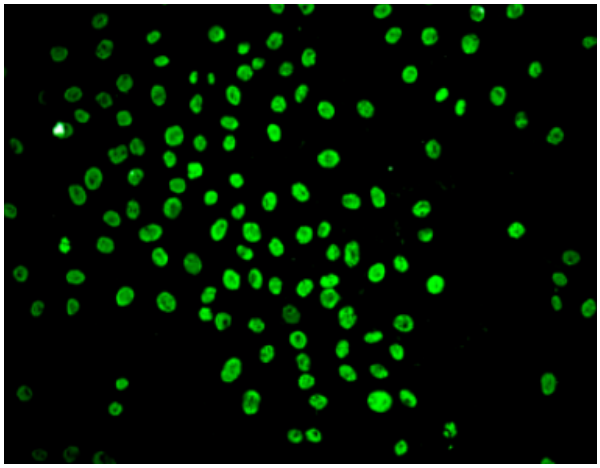

FIGURE // 1B

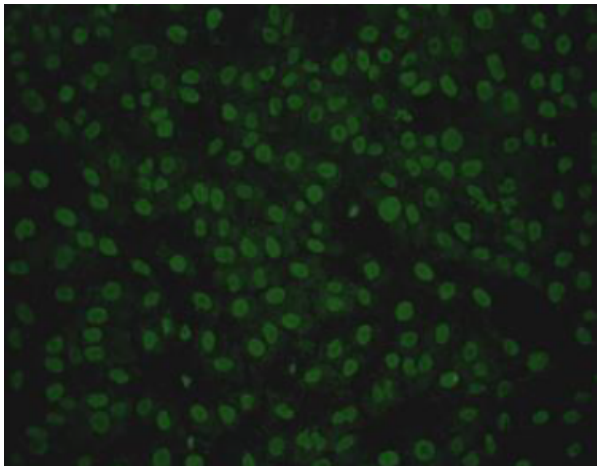

Figure 1: H_2O_2 treated (A) and untreated (B) MCF-10A cells stained with 8-oxo-dG Antibody (catalog# 4354-MC-050) according the protocol using an Alexa Fluor 488 conjugated anti-mouse antibody.

Immunohistochemistry Protocol

1. Deparaffinize tissue sections three times in xylene for 5 minutes each.

2. Rehydrate sequentially each 3 minutes, twice in the following ethanol solutions: A, 100%; B, 95%; C, 80%; D, 50%. Rinse with distilled water.

3. Wash twice in 1X PBS for 2 minutes each.

Note: Antigen retrieval is necessary only to stain the tissues for other antigens, it is not necessary for detection of 8-oxo-dG alone.

4. Cover sections with 200 µL of Proteinase K in 1X PBS and incubate for 15-30 minutes at 37 °C.

5. Wash sections in 1X PBS for 5 minutes each.

Note: RNase treatment is optional but recommended in studies of mitochondrial oxidation to increase the sensitivity and specificity of the detection method. For RNA oxidation studies, omit the RNase step. The antibody recognizes 8-oxo-G in RNA as well as in DNA.

6. Incubate sections in 200 µL of buffer containing 100 µg/mL RNase A, 150 mM NaCl, and 15 mM sodium citrate for 1 hour at 37 °C.

7. Wash twice with 1X PBS for 5 minutes each.

8. Denature the DNA by treating the slides with 2N HCl for 5 minutes at room temperature.

9. Neutralize the sample by soaking the slides in 1M Tris-base for 5 minutes at room temperature.

10. Wash twice in 1X PBS for 5 minutes each.

11. Block non-specific binding sites by incubating the tissue sections in 10% normal goat serum in PBS for 1 hour at room temperature.

12. Aspirate blocking solution.

13. Incubate sections 250 µL of a 1:250 dilution of the primary antibody in 1X PBS containing 0.1% BSA, o/n at 4 °C. Incubate control sections in antibody diluent or with an isotype matching control in a humidified chamber. Titration of the antibody is advised to obtain optimal results.

14. Wash sections three times with 1X PBS for 3 minutes each.

15. Incubate all sections with 250 µL of goat-anti mouse secondary antibody (Alexa Fluor 488) at 5 µg/mL in 1X PBS containing 0.1% BSA **in the dark** at room temperature.

16. Rinse and wash with 1X PBS four times for 3 minutes each.

17. Rinse sections in water and counter stain with 1:50 in water of 7AAD solution (40 µg/mL). Apply a sufficient amount to cover sections. Incubate **in the dark** for 30 minutes at room temperature.

Note: 7AAD-Molecular Probes-546 nm excitation/ 647 nm emission; an option would be to use DAPI provided the microscope of use is equipped with the appropriate filters.

18. Rinse twice and wash twice with 1X PBS for 5 minutes each.

19. Mount with appropriate mounting medium and image by fluorescence microscopy.

Example Results

FIGURE // 2 // 8-OXO-DG MONOCLONAL ANTIBODY STAINING OF A RAT THYMUS SECTION

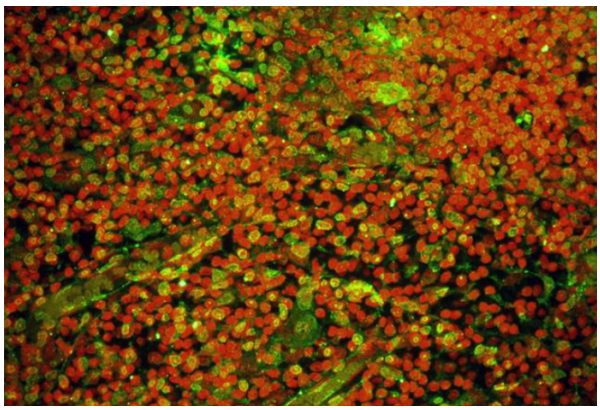

Figure 2: Paraffin embedded rat thymus sections were stained with 8-oxo-dG monoclonal antibody (catalog# 4354-MC-050) at a 1:250 dilution and detected by Alexa-488, which appears fluorescent green. 8-oxo-dG is present in the cytoplasm and nucleus of most but not all cells. Sections were counter-stained with 7-Aminoactinomycin D (7AAD), which labels nuclear DNA. Images were captured at 40X magnification.