



Product Data

For Research Use Only. Not For Use In Diagnostic Procedures
Anti-8-oxo-dG

Catalog #: 4370-096-05 **Volume:** 50 µl

Description: This mouse monoclonal antibody specifically binds to 8-hydroxy-2'-deoxyguanosine within DNA in H₂O₂-treated cells. It can be used to detect oxidative damage by ELISA (Trevigen Cat# 4370-096-K) 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 in 1X PBS containing 0.01% sodium azide.

Ig Class: IgG_{2b}

Storage Conditions: This antibody can be stored at -20 °C or -80 °C. Avoid repeated freeze-thawing by aliquoting into smaller portions.

Applications: Immunodetection of 8-oxo-dG by ELISA and immunocytochemistry. 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.

Immunocytochemistry Protocol:

1. Plate cells 5x 10⁴ cells (sub-confluent) on cover slips or chamber slides o/n
2. Aspirate medium, wash cells with 1X PBS, and treat with 300µl of 100-300µM H₂O₂ 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 0.05N HCl for 5 minutes on ice.
5. Wash 3x with 1X PBS, 5 minutes each.
6. Incubate with 250µl of 100µg/ml RNase in 150mM NaCl, 15mM 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 250µl 0.15N NaOH in 70% EtOH for 4 minutes.
9. Wash briefly 2x with 1X PBS.
10. Use 0.2 µg/ml (250µl) Hoechst 33342 (Immunochemistry Technologies, LLC) 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 250µl of 5µg/ml proteinase K in 20mM Tris, 1mM EDTA, pH 7.5 (TE) for 10 minutes at 37 °C.
13. Wash several times with 1X PBS.
14. Block non-specific binding with 5% normal goat serum in 1X PBS, 1hour at RT.
15. Wash 3x with 1X PBS, and incubate with 250µl 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.

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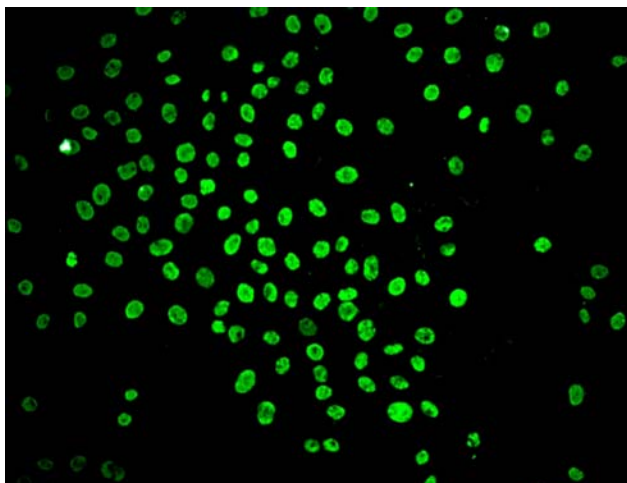
16. Wash several times with 1X PBS containing 0.05% Tween 20 for 5 minutes each.
17. Incubate cells in 250µl of fluorescent secondary antibody conjugate, goat antimouse IgG (Alexa Fluor 488 (Molecular Probes)) at 5µg/ml in 1X PBS containing 1% BSA for 1hr in the dark, at room temperature.
18. Wash several times with 1X PBS containing 0.05% Tween 20.
19. Rinse with de-ionized water.

20. Mount with appropriate mounting media e.g. Permount.

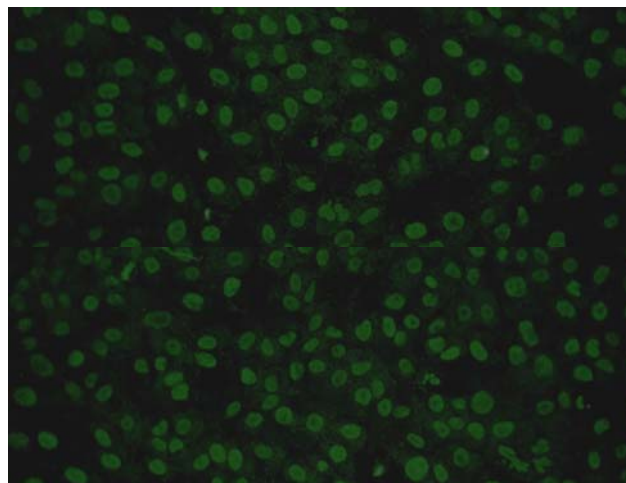
Note:

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 Result:



A



B

Reference:

1 Soutanakis RP, Melamede RJ, Bespalov IA, Wallace SS, Beckman KB, Ames BN, Taatjes DJ, Janssen-Heininger YMW. (2000) Fluorescence detection of 8-oxoguanine in nuclear and mitochondrial DNA of cultured cells using a recombinant Fab and confocal scanning laser microscopy. Free Rad Biol Med 28:987-998.

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1-800-873-8443

H₂O₂ treated (A) and untreated (B) MCF-10A cells stained with 8-oxo-dG antibody (Cat# 4370-096-05) according to the above protocol using an AlexaFluor 488 conjugated anti-mouse antibody.

