TREVIGEN® Product Data

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Anti-8-oxo-dG (15A3)

Catalog #: 4354-MC-050 **Volume:** 50 µl

Description: This mouse monoclonal antibody specifically binds to 8-hydroxy-2'-deoxyguanosine within DNA in H_2O_2-treated cells. It can be used to detect oxidative damage by ELISA (cat# 4380-096-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 at 0.5 mg/ml in 1X PBS containing 0.1% sodium azide, 50% glycerol.

Ig Class: IgG_{2b}

Storage Conditions: -20°C

Applications: Immunodetection of 8-oxo-dG by ELISA, immunocytochemistry, and immunofluorescence. 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 5x 10^4 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 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 0.05N 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 anti-mouse 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 Results:

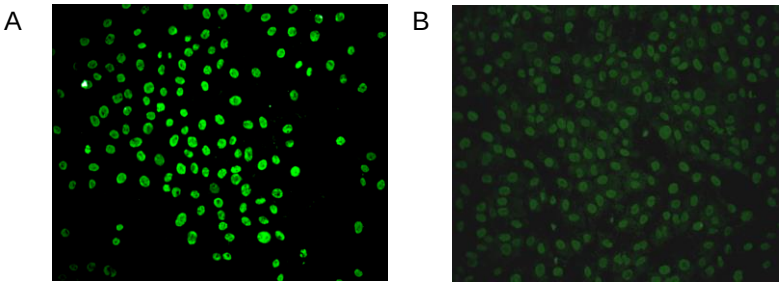

Figure 1: H_2O_2 treated (A) and untreated (B) MCF-10A cells stained with 8-oxo-dG antibody (cat# 4354-MC-050) according to the above protocol using an Alexa Fluor 488 conjugated anti-mouse antibody.

8-oxo-dG Monoclonal Antibody Staining of a Rat Thymus Section

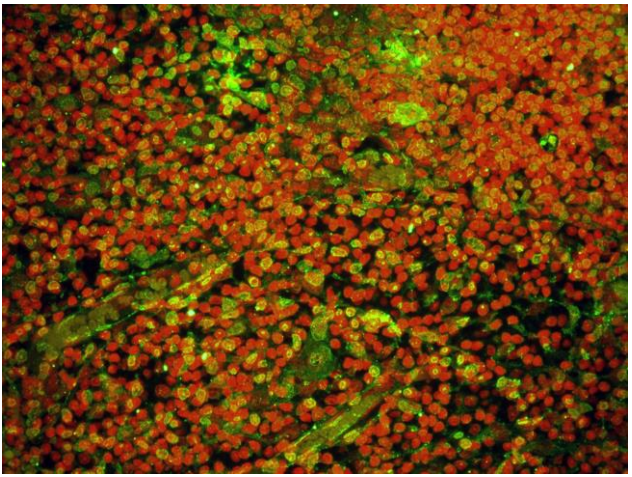

Figure 2. Paraffin embedded rat thymus sections were stained with 8-oxo-dG monoclonal antibody (cat# 4354-MC-050) at a 1:250 dilution and detected by Alexa-488, which appears fluorescent green. 8-oxo-dG is present in the cytoplasm and nucleus of most but not all cells. Sections were counter-stained with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

Protocol for IHC with 8-Oxo-dG antibody on paraffin embedded rat thymus tissue (figure 2):

1. Deparaffinize tissue sections thrice 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 (2 minutes each).

Note: antigen retrieval is necessary only if you want to stain the tissues for other antigens too, 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 ^{o}C.
5. Wash sections in 1X PBS, 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 1h at 37 ^{o}C.
7. Wash twice with 1X PBS, 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 (5 minutes each).
11. Block non-specific binding sites by incubating the tissue sections in 10% normal goat serum in PBS, 1h 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 ^{o}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, 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 dark at room temperature.
16. Rinse and wash with 1X PBS four times, 3 minutes each.
17. Rinse sections in water and counter stain with 1:50 in water of 7AAD solution (40ug/ml). Apply a sufficient amount to cover sections. Incubate in the dark for 30 minutes at room temperature. *
18. Rinse twice and wash twice with 1X PBS for 5 minutes each.
19. Mount with appropriate mounting medium and image by fluorescence microscopy.

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

Reference:

1. Soultanakis RP, Melamede RJ, Bespalov IA, Wallace SS, Beckman KB, Ames BN, Taatjes DJ, Janssen-Heininger YMW. (2000) Flourescence 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.

Related DNA Damage Antibodies:

Catalog #	Description	Size
4418-APC-020	γ-H2AX polyclonal antibody	20 µl
4418-APC-100	γ-H2AX polyclonal antibody	100 µl
4335-AMC-050	Anti-PAR mAb (clone 10HA) affinity purified	50 µl
4335-MC-100	Anti-PAR mAb (clone 10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg