QED Bioscience Inc.

Immunostaining with 8-OHdG Monoclonal Antibody (catalog no. 12501)

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 overnight.

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.