Immunohistochemistry Protocol for ab62623

Tissue Preparation:
ab62623 reacts on both 50 µm frozen tissue sections and paraffin-embedded sections. Tissue should be dissected fresh and fixed in periodate-lysine-paraformaldehyde (PLP) at 4°C overnight.

PLP
- Heat 1 L dH_2O to 60°C.
- Add 60 g paraformaldehyde.
- Add 33 g dibasic $NaPO_4$.
- Cool to room temperature 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 cryo-protected 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

0.1M Phosphate Buffer, pH 7.4 (for 1 L)
- 1L 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 de-paraffinized 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. Pre-treat sections with a methanol-peroxide solution to eliminate endogenous peroxidases.

Methanol-Peroxide
- 100 ml absolute methanol
- 1 ml 33% H2O2

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 µg/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 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) 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°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

Immunocytochemistry Protocol for ab62623

- Grow cells on glass microscope slides, glass cover slips or slide culture chambers. Remove culture medium and gently wash cells 3 times with ice cold PBS. Fix cells by adding a volume of 4% formaldehyde in PBS equal to the original volume of culture medium for 30 minutes on ice. Remove the fixative and wash 3 times for 5 minutes each with PBS. If desired, incubate 5 minutes in 1% H_2O_2 in PBS to remove endogenous peroxidase activity. Wash the fixed cells 3 times for 5 minutes each with PBS.
- Prepare appropriate dilution of primary antibody by diluting in Antibody Dilution Buffer. Remove the buffer from the cells. Add a sufficient volume of diluted primary antibody to cover the cells. Incubate with primary antibody for 60 minutes at room temperature. If the primary antibody has a low-affinity for the antigen, incubate at 4ºC overnight. Remove primary antibody solution. Wash 3 times for 5 minutes each with PBS.
- Remove the buffer from the cells. Add diluted Biotinylated Secondary Antibody and incubate for 30 minutes at room temperature. The optimum dilution may be lot specific. Remove solution. Wash 3 times for 5 minutes each with PBS.
- Remove the buffer from the cells. Add Diluted Streptavidin Peroxidase and incubate for 30 minutes at room temperature. Remove solution. Wash 3 times for 5 minutes each with PBS.
- Remove buffer. Add DAB substrate and incubate approximately 10 minutes or until sufficient color develops.
- Remove solution. Wash 3 times for 2 minutes each with distilled water. Counter stain with hematoxylin for 1 to 5 minutes depending on the concentration and color intensity desired. Wash 3 times for 2 minutes each with distilled water. Dehydrate the cells with 100% ethanol 4 times for 2 minutes each. Clear the cells with xylene 4 times for 2 minutes each. Add 2-3 drops of mounting media add a cover slip and allow to air dry.
- Observe cells under the microscope. A positive reaction should be visible as a brown precipitate. The nuclei should appear light blue.