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NBP2-75923 Protocol

Immunohistochemistry-Paraffin protocol for Ferroportin/SLC40A1 Antibody (NBP2-75923)

Ferroportin/SLC40A1 Antibody (1308C): https://www.novusbio.com/products/ferroportin-slc40a1-antibody-1308c_nbp2-75923
Recommended Protocol for IHC and ICC Staining

1. Deparaffinize paraffin-embedded sections in Xylene and hydrate in a series of graded alcohol to water. Perform Heat Induced Epitope Retrieval (HIER) if required. Rinse with deionized water. For frozen tissue sections deparafinization is not required. Cells can be fixed in dishes they are cultured in, but don't require heat-induced epitope retrieval: for example, cells can be fixed with freshly made 2-4% formaldehyde solution for 20 minutes at room temperature and then rinsed 3 x 10 minutes with PBS. Do not let tissue sections or cells dry from this point on.
2. Block endogenous peroxidase by incubating slides with tissue sections with 3% H2O2/Methanol solution for 15 minutes at room temperature.
3. Rinse the sample 3 times with PBS containing 0.05% Tween 20.
4. Block with normal serum of choice, by incubating slides for 15 minutes at room temperature.
5. Drain or blot off solution, but do not rinse.
6. Apply primary antibody and incubate for 60 minutes at room temperature. It is the responsibility of the investigator to optimize working dilution and incubation time for primary antibodies.
7. Repeat step 3.
8. Apply the VisUCyte(TM) HRP Polymer, covering the entire area of the tissue sections, and incubate for 30 to 60 minutes at room temperature.
9. Repeat step 3.
10. Add HRP-sensitive substrate solution (which produces insoluble precipitate) to tissue sections and incubate using conditions recommended by the substrate's supplier. For example, HRP-sensitive substrate solution can be either, 3' 3'-Diaminobenzidine (known as DAB) 3-Amino-9-ethylcarbazol (known as AEC).
11. Rinse with distilled tap water.
12. Counterstain tissue sections if required using hematoxylin or other counterstaining dyes as needed for 15 seconds to 2 minutes depending on the tissue.
13. Rinse slides with tap water (if hematoxylin was used) or other solution as required for the particular counterstaining dye and mount tissue sections under coverslips with either aqueous or non-aqueous (e.g. xylene-based) mounting media. Note: Unlike DAB, AEC is soluble in alcohols and xylene. Tissue sections subjected to an HRP-AEC protocol should be coverslipped using only aqueous mounting media.
14. Let slides dry and visualize staining under a microscope.

Precautions
Avoid using preservatives in solutions, such as Sodium Azide which is a strong inhibitor of HRP enzyme.