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NLS8182 Protocol

Immunohistochemistry protocol for GPR56 Antibody (NLS8182)

Immunohistochemistry Protocol for GPR56 Antibody (NLS8182): https://www.novusbio.com/products/gpr56-antibody_nls8182
Immunohistochemistry

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
2. Make 4-um sections and place on pre-cleaned and charged microscope slides.
3. Heat in a tissue-drying oven for 45 minutes at 60 degrees Celcius.
4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene approximately 5 minutes each @ RT.
5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol approximately 3 minutes each @ RT.
6. Wash the slides in 2 changes of 95% alcohol approximately 3 minutes each @ RT.
7. Wash the slides in 1 change of 80% alcohol approximately 3 minutes @ RT.
8. Rinse the slides in gentle running distilled water approximately 5 minutes @ RT.
9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
11. Rinse the slides in 1X TBS-T for 1 minute @ RT.

Do not allow the tissues to dry at any time during the staining procedure

12. Begin the immunostaining by applying a universal protein block approximately 20 minutes @ RT.
13. Drain protein block from the slides and apply the diluted primary antibody approximately 45 minutes @ RT.
14. Rinse the slide in 1X TBS-T approximately 1 minute @ RT.
15. Apply a biotinylated anti-rabbit IgG (H+L) secondary approximately 30 minutes @ RT.
16. Rinse the slide in 1X TBS-T approximately 1 minute at RT.
17. Apply an alkaline phosphatase steptavidin approximately 30 minutes at RT.
18. Rinse the slide in 1X TBS-T approximately 1 minute at RT.
19. Apply an alkaline phosphatase chromagen substrate approximately 30 minutes at RT.
20. Rinse the slide in distilled water approximately 1 minute @ RT.

This method should only be used if the chromagen substrate is alcohol insoluble (ie: Vector Red, DAB)

21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol approximately 1 minute each @ RT.
22. Wash the slides in 2 changes of 95% alcohol approximately 1 minute each @ RT.
23. Wash the slides in 3 changes of 100% alcohol approximately 1 minute each @ RT.
24. Wash the slides in 3 changes of xyleneapproximately 1 minute each @ RT.
25. Apply cover slip.