

## NLS6324 Protocol

### Immunohistochemistry protocol for GPR88 Antibody (NLS6324)

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Immunohistochemistry

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
2. Make 4-mm 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 5 minutes each at RT.
5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol 3 minutes each at RT.
6. Wash the slides in 2 changes of 95% alcohol 3 minutes each at RT.
7. Wash the slides in 1 change of 80% alcohol 3 minutes at RT.
8. Rinse the slides in gentle running distilled water 5 minutes at RT.
9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) at 99-100 degrees Celcius  
for 20 minutes.
10. Remove the slides from the heat and let stand in buffer at RT for 20 minutes.

11. Rinse the slides in 1X TBS-T for 1 minute at 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 20 minutes at RT.
13. Drain protein block from the slides and apply the diluted primary antibody 45 minutes at RT.
14. Rinse the slide in 1X TBS-T 1 minute at RT.
15. Apply a biotinylated anti-rabbit IgG (H+L) secondary 30 minutes at RT.
16. Rinse the slide in 1X TBS-T 1 minute at RT.
17. Apply an alkaline phosphatase streptavidin 30 minutes at RT.
18. Rinse the slide in 1X TBS-T 1 minute at RT.
19. Apply an alkaline phosphatase chromagen substrate 30 minutes at RT.
20. Rinse the slide in distilled water 1 minute at 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 1 minute each at RT.
22. Wash the slides in 2 changes of 95% alcohol 1 minute each at RT.

23. Wash the slides in 3 changes of 100% alcohol 1 minute each at RT.
24. Wash the slides in 3 changes of xylene 1 minute each @ RT.
25. Apply cover slip