Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NLS1595

NLS1595 Protocol

Protocol specific for GPCR GPR40 Antibody (NLS1595)

Nuclear Extract and Cytoplasmic Fraction Preparation protocol for GPCR GPR40 Antibody (NLS1595):
https://www.novusbio.com/products/ffar1-gpr40-antibody_nls1595
Procedure Guide for NLS 1595 - G Protein-Coupled Receptor GPR40

Antibody

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 @ 60 degrees Celcius.

4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene alpha 5 minutes each @ RT.

5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol alpha 3 minutes each @ RT.

6. Wash the slides in 2 changes of 95% alcohol alpha 3 minutes each @ RT.

7. Wash the slides in 1 change of 80% alcohol alpha 3 minutes @ RT.

8. Rinse the slides in gentle running distilled water alpha 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 alpha 20 minutes @ RT.

13. Drain protein block from the slides and apply the diluted primary antibody alpha 45 minutes @ RT.

14. Rinse the slide in 1X TBS-T alpha 1 minute @ RT.

15. Apply a biotinylated anti-rabbit IgG (H+L) secondary alpha 30 minutes @ RT.

16. Rinse the slide in 1X TBS-T alpha 1 minute @ RT.

17. Apply an alkaline phosphatase steptavidin alpha 30 minutes @ RT.

18. Rinse the slide in 1X TBS-T alpha 1 minute @ RT.

19. Apply an alkaline phosphatase chromagen substrate alpha 30 minutes @ RT.

20. Rinse the slide in distilled water alpha 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 alpha 1 minute each @ RT.

22. Wash the slides in 2 changes of 95% alcohol alpha 1 minute each @ RT.

23. Wash the slides in 3 changes of 100% alcohol alpha 1 minute each @ RT.

24. Wash the slides in 3 changes of xylene alpha 1 minute each @ RT.

25. Apply cover slip.