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NB300-119 Protocol

Western Blot Protocol for Neurokinin 1 Receptor Antibody (NB300-119)

[[URL:https://www.novusbio.com/products/nk1r-antibody_nb300-119]][[Caption:Neurokinin 1 Receptor Antibody]]
Western Analysis

1) Separate 50 ug of whole cell or membrane protein on a 4-20% Tris-glycine gel.
2) Transfer the proteins from the gel onto a nitrocellulose membrane.
3) Incubate the nitrocellulose membrane in Blocking solution [5% non-fat dry milk / PBS] overnight at 4 degrees C.
4) Incubate the membrane with a 1:1,000 dilution of anti-NK-1 antibody (Novus Biologicals, Inc., #NB300-119) in PBST-NFDM [PBS / 0.1% Tween-20 / 5% non-fat dry milk] for 2 hours at RT.
5) Wash the membrane with PBST-NFDM.
6) Incubate the membrane with HRP-conjugated goat anti-rabbit IgG diluted with PBST-NFDM for 1 hour at RT.
7) Detect antibody with an enhanced chemiluminescence reaction (ECL Western Blotting detection kit, Pharmacia).

FACS Analysis

1. Seed cells into small (T-25) flask. Add 5-10 ml media. Allow cells to grow to 80-100% confluence.
2. When flasks are confluent, empty media and add new media containing appropriate drug, hormone, or cytokine dose.
3. Allow flasks to incubate for 24 hours. After incubation period, remove 2 ml of media and place 1 ml into one of two eppendorf tubes.
4. Empty off remaining media. Wash with 5 ml 1X PBS. 5. Add 2 ml PBS with azide (1% Goat serum).
5. Scrape cells using a cell scraper.
6. Transfer cells to appropriate labeled FACSCAN tubes. Vortex all tubes
7. Incubate on ice for - hour in the PBS with azide (1% Goat serum). Vortex all tubes after incubation.
8. Centrifuge at 1400 RPM for 5 minutes. Empty off media.
9. Resuspend in 1 ml of a 1:3,000 primary antibody solution (NB 300-119) [1 ul antibody per 3 ml PBS with azide (1% Goat serum)].
10. Incubate on ice for 1 hour. Vortex all tubes after incubation.
11. Centrifuge at 1400 RPM for 5 minutes. Aspirate (or decant) supernatant.
12. Wash twice with 2 ml ice-cold PBS with azide (1% Goat serum).
13. Aspirate (or decant) supernatant and place tubes on ice.
14. Resuspend in 1 ml of a 1:500 secondary antibody solution [2 ul antibody per 1 ml PBS with azide (1% Goat serum)].
15. Vortex all tubes.
16. Cover with foil. Incubate for 30 minutes on ice.
17. Vortex after incubation. Centrifuge at 1400 RPM for 5 minutes.
18. Aspirate (or decant) supernatant. Wash twice with 2 ml ice-cold PBS with azide (1% Goat serum).
19. Aspirate (or decant) supernatant. Resuspend in 2% paraformaldehyde.
20. Read on flow.
FACS Analysis procedure courtesy of: Rosalyn Blumenthal, Ph.D. Director Tumor Biology Garden State Cancer Center Belleville, NJ 07109

IHC-FFPE sections

I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.

Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).