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NB400-132 Protocol

Protocol specific for ABCG1 Antibody (NB400-132)

Western Blot Protocol for NB 400-132
Protein Extraction:
1. After washing with PBS, cells (mouse peritoneal macrophages grown in a 60 mm dish) in 300 ml of cold lysis buffer [50 mM Tris, pH 7.5, 100 mM NaCl, 1% Triton x-100, 1% NaC24H39O4, 50 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 50 ug/ml of aprotinin and 50 ug/ml of leupeptin] are lysed by scraping and sonicating for 25 seconds on ice.
2. Spin cellular lysate for 10 min. at 13,000 rpm at 4 degrees Celcius.
3. Save supernatant and store at -20 degrees Celcius.
Western blotting:
1. Determine protein content by Lowry method.
2. Load 40 ug of cellular protein [pre-boiled for 5 min. in sample buffer] on a 7.5% SDS-PAGE separating gel.
3. Run electrophoresis for 90 min. at RT in 1x electrophoresis buffer.
4. After electrophoresis, equilibrate the gel and nitrocellulose membrane in transfer buffer.
5. Transfer proteins in 1x transfer buffer for 1 hour at 100 volts and RT.
6. Block the membrane in 10 ml of TBS with 5% NFDM for 1 hour at RT.
7. After a quick rinse with TBS-T (0.5% Tween-20), membrane is incubated in diluted anti-ABCG1 (cat# NB 400-132) in 1% NFDM/TBS for 1.5 hours at RT.
8. Wash the membrane in 25 ml of TBS-T for 3x 5 minutes at RT.
9. Incubate the membrane in 10 ml of diluted secondary antibody (Anti-Rabbit IgG-HRP Conjugate) in 1% NFDM/TBS for 1 hour at RT.
10. Wash the membrane with 25 ml of TBS-T for 3x 5 minutes at RT.
11. Incubate the membrane in ECL Western blotting detection reagents for 1 minute.
12. Expose to film for ~2 minutes (adjust time as needed for best image).
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).