

## NB400-132 Protocol

# Western Blot protocol for ABCG1 Antibody (NB400-132)

ABCG1 Antibody: [https://www.novusbio.com/products/abcg1-antibody\\_nb400-132](https://www.novusbio.com/products/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% NaC<sub>24</sub>H<sub>39</sub>O<sub>4</sub>, 50 mM NaF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 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).