

NB400-105 Protocol**Western Blot protocol for ABCA1 Antibody (NB400-105)****Western Blot**

The experiment was performed by treating RAW macrophages with 9-cis-retinoic acid and 22R-hydroxycholesterol, known inducers of ABCA1 expression in macrophages. Then total cell post-nuclear lysate (40ug protein) was separated by SDS-PAGE and detected using a 1:1000 dilution of NB 400-105 affinity purified Lot G incubated for 1 hour at room temperature (Lane A). Although there are lower molecular weight bands on the blot, the ABCA1 signal is excellent and gives the expected 3 bands. It is not known why ABCA1 runs as three bands, but it has been found to do so by many researchers. It is probably due to protein modifications such as glycosylation. The antibody was also tested against ABCA1 transiently expressed in 293 cells as an independent test with excellent results.

NOTE: An important factor in detecting ABCA1 is in the cell type used. ABCA1 is expressed in very low levels in most cell types. Therefore, ABCA1 expression needs to be induced by using 22-hydroxycholesterol and 9-cis-retinoic acid as ligands for the transcription factor LXR.

1. Without heating at all (leave at room temp for about 15 to 20 minutes with Beta-mercaptoethanol), load 40 ug post-nuclear lysates* to 7.5% or 4-15% Tris-HCL SDS gel (Bio-RAD) in sample buffer. Do NOT boil the samples. (NP-40 will not interfere with the running of the protein on SDS-PAGE.)

2. Transfer to nitrocellulose membrane at 100V 1hr or 30V overnight.

3. Block membrane in 5% milk in TBS-T for at least 1 hr. Wash with TBS-T 5 minutes.

4. Blot with anti-ABCA1 antibody in 3% milk in TBS-T for 1 hour.

5. Wash with TBS-T 3 times, 10 minutes each.

6. Blot with donkey anti-rabbit secondary; antibody (Amersham) at 1:2000 in 3% milk in TBS-T for 1 hour.

7. Wash with TBS-T 3 times, 10 minutes each.

8. Detect with chemiluminescent reagent (Pierce).

9. Expose to X-ray film, 30 sec., 1 min., and 3 min. TBS-T: Tris-buffered-saline with Tween-20

See also the specific references mentioned in the datasheet. *Post-nuclear lysate is the result of sonication or dounce homogenization of lysate, centrifugation at low-speed, and the removal of nuclei. The resulting supernatant is called post-nuclear and contains cytosolic and membrane proteins without any of the nuclear components.