RAW macrophages were treated with 9-cis-retinoic acid and 22R-hydroxycholesterol, known inducers of ABCA1 expression in macrophages. The total cell post-nuclear lysate (40ug protein) was separated by SDS-PAGE and detected using a 1:1000 dilution of NB400-105 affinity purified ABCA1 antibody incubated for 1 hour at room temperature. ABCA1 has been found to run as 3 bands by many researchers; this is probably due to protein modifications such as glycosylation.

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 anti-rabbit secondary according to the recommended dilutions 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. Image

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.