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NB400-101 Protocol

Western Blot protocol specific for SR-BI Antibody (NB400-101)

Procedure Guide for NB400-101 Polyclonal anti-SR-B1

Western Blot Procedure

1. Run ~50 ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 60 minutes.
2. Equilibrate gel, nitrocellulose membrane, Whatman paper, and blotting pads in transfer buffer for 15 minutes.
3. Transfer protein to the membrane at 25V for 90 minutes.
4. Allow membrane to air-dry.
5. Block membrane with 1XPBS/5% non-fat milk/0.1% Tween-20 for 1 hour at room temperature (~23-27 degrees C).
6. Wash membrane twice, for 5 minutes each, with 1XPBS/0.05% Tween-20 (PBST).
7. Incubate membrane with 1:1,000 dilution of NB400-101 (anti-SR-BI), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
9. Incubate membrane with 1:10,000 dilution of goat anti-rabbit IgG-HRP (BioRad), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
10. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
11. Detect cross-reacting proteins using Renaissance Chemiluminescence Reagent Plus kit from NEN Life Sciences.

*NOTE: HL-60 whole cell extracts (NB800-PC3) were used as a positive control for this antibody.