Western Blot protocol for Nbs1 Antibody (NB100-143)

Western Blot Procedure

1. Run ~50ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 90 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/3% BSA 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 NB100-143 (anti-hp95/nibrin), 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 goat anti-rabbit IgG-HRP, 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.

Note: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.