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NB300-164 Protocol

Western Blot protocol for Bestrophin 1 Antibody (NB300-164)

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Procedure Guide for NB 300-164 Monoclonal Anti-Bestrophin

Western Blot Procedure

1. Run cell lysates** on an SDS-PAGE gel.
2. Transfer the proteins to PVDF.
3. Block the membrane in 1% Carnation instant milk in PBS + 0.1% Tween 20 (with 0.1mM CaCl2 and 1mM MgCl2) for 1 hour at RT.
4. Dilute the anti-Bestrophin [NB 300-164] to 1:1,000 in 10 ml of fresh blocking buffer and incubate for 1 hour at RT.
5. Wash the membrane with blocking buffer, 3x 5-10 minutes.
6. Dilute the secondary antibody in fresh blocking buffer, as recommended by the secondary vendor and incubate for 1hour at RT.
7. Wash the membrane with blocking buffer, 5x 8 minutes and rinse 1x with PBS (containing 0.1mM CaCl2 and 1mM MgCl2).
8. Detect the protein-antibody complex with alkaline phosphatase, if using NBT/BCIP or with HRP, if using ECL.

**Cell Lysate Preparation
A. Lysates were prepared in lysis buffer [50mM Tris-HCl, pH 8 / 120mM NaCl / 0.5% Nonidet P-40 / 10 ug/ml aprotinin / 10 ug/ml leupeptin / 1mM phenylmethylsulfonyl fluoride / 1mM sodium orthovanadate].
B. Total protein content was determined by bicinchoninic acid assay (Pierce).