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NB300-144 Protocol

Western Blot protocol for Laminin Antibody (NB300-144)

[[URL:https://www.novusbio.com/products/laminin-antibody_nb300-144]][[Caption:Laminin Antibody]]
Western Blot
1. SDS-PAGE on 5% mini-gel under reducing conditions
2. Electroblot to nitrocellulose by Towbin methods
3. Remove nitrocellulose sheet from electroblotting sandwich and rinse briefly in dH2O.
4. Fixation: In a glass dish immerse the blot in 25% isopropanol/10% acetic acid/ 65% dH2O. Cover and shake gently for at least 30 min. at room temperature.
3. Remove the blot from fixative and wash in a large volume changes of dH2O for > 10 min.
4. Place blot in plastic tray with lid. Equilibrate >10 min. with Washing Buffer. Pour off.
5. Blocking: Place the blot in Blocking Buffer (just enough to cover). Incubate with gentle shaking for at least 1 h (overnight if background is a big problem). Pour off.
6. Primary Antibody: Dilute PcAbLN antibody (3/4 1 ug/ml) in Blocking Buffer. Add just enough to cover blot and incubate with shaking for 2 h at 37C or overnight at room temp.
7. Pour off primary antibody and wash X3 with Washing Buffer over 20-30 min (or more).
8. Peroxidase-conjugated Secondary Antibody: Dilute peroxidase conjugated 2 degrees IgG (Dako, affinity purified) diluted 1:2000 in Blocking Buffer. Add just enough to cover the blot and incubate with shaking for 2 h at 37C.
9. Wash blot thoroughly (30 min and up to hours) in Washing Buffer and then with a final wash in Washing buffer without Triton.
10. Chemiluminescent Detection: According to manufacturers instructions.

Washing Buffer
0.05 Tris-HCl, pH 7.4
1.5% NaCl
0.1% Triton X100

Blocking Buffer
Washing Buffer
5% powered milk
(dissolve for hours, filter)