

NB400-118 Protocol

Protocol specific for Endothelial Lipase Antibody (NB400-118)

Western Blot Procedure

1. Heat diluted samples at 70 degrees Celcius for 10 minutes.
2. Load samples onto a 10% Bis-Tris gel.
3. Run gel for ~45 minutes at 200V.
4. Transfer gel to PVDF membrane at 45V for ~90 minutes.
5. Block the membrane overnight at 4 degrees Celcius, in blocking buffer [PBS + 0.2% Tween-20 + 5% milk], gently shaking.
6. Rinse the membrane twice, quickly, in PBS-T.
7. Wash the membrane in PBS-T 3 times for 5 minutes, each.
8. Incubate the membrane with primary antibody [NB 400-111 or NB 400-118] , diluted 1:500 in blocking buffer, for 1 hour at RT, gently shaking.
9. Rinse the membrane twice, quickly, in PBS-T.
10. Wash the membrane in PBS-T 3 times for 5 minutes, each.
11. Incubate the membrane with secondary antibody [NB 730-H], diluted 1:5,000 in blocking buffer, for 1 hour at RT, gently shaking.
12. Rinse the membrane twice, quickly, in PBS-T.
13. Wash the membrane in PBS-T 3 times for 5 minutes, each.
14. Detect protein using ECL, ~1 minute exposure.