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## NBP2-36496 Protocol

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Western Blot protocol for Thrombomodulin/BDCA-3 Antibody (NBP2-36496)

Thrombomodulin/BDCA-3 Antibody (4D8.2C6): https://www.novusbio.com/products/thrombomodulin-bdca-3-antibody-4d82c6\_nbp2-36496
Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
- 2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
- 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
- 4. Rinse the blot.
- 5. Block the membrane using standard blocking buffer for at least 1 hour.
- 6. Wash the membrane in wash buffer three times for 10 minutes each.
- 7. Dilute anti-Thrombomodulin primary antibody in blocking buffer and incubate 1 hour at room temperature.
- 8. Wash the membrane in wash buffer three times for 10 minutes each.
- 9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
- 10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
- 11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.