

NB300-232 Protocol

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Western Blot Protocol for SCP3/SYCP3 Antibody (NB300-232)

## Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
- 2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
- 3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
- 4. Rinse the blot TBS -0.05% Tween 20 (TBST).
- 5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
- 6. Wash the membrane in TBST three times for 10 minutes each.
- 7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
- 8. Wash the membrane in TBST three times for 10 minutes each.
- 9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
- 10. Wash the blot in TBST 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.