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## NB100-129 Protocol

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## Western Blot Protocol for BMAL1 Antibody (NB100-129)

Procedure Guide for NB 100-129 (Anti-MOP3 aka Anti-BMAL1)

Western Blot Procedure

- 1. Proteins are separated by electrophoresis.
- 2. Proteins are transferred to nitrocellulose sheets.

3. Following the protein transfer, the filters are blocked for 1 hour at room temperature, gently shaking.

4. The filters are then incubated with a 1:100 dilution of (NB100-129) anti-MOP3 (aka: Banti-BMAL1) in PBS + 5% Dry Milk for 2 hours at room temperature, gently shaking. \*

5. The filters are washed three times with TBST at room temperature.

6. The filters are then incubated with an Alkaline Phosphatase conjugated secondary antibody [Goat anti-Rabbit Alk Phos conjugated Ab from Jackson ImmunoResearch #111-055-003], diluted 1:5000 in PBS + 5% Dry Milk, for 1 hour at room temperature.

- 7. The filters are washed three times with TBST at room temperature.
- 8. Stained the filters with NBT/BCIP substrate at room temperature.
- 9. The filters are finally washed three times with TBST at room temperature.
- \* Overnight at 4 degrees C will increase sensitivity