

## Western blots for mouse incisor mesenchymal cells

### Day 1

1. Loading and running the gel

Load protein extracts (20–30  $\mu$ g) from the mouse incisor in 4%-15% precast polyacrylamide gel (Bio-Rad, 456-1084) and run at 110V for 60min.

2. Transferring the protein from the gel to the membrane

Activate PVDF with methanol for 1 min and rinse with transfer buffer before preparing the stack. Current: 300mA; time of transfer: 90min.

3. Block with 5% milk (Bio-Rad,170-6404) for 1h, and incubate with primary antibody with appropriate dilutions overnight at 4°C.

### Day 2

4. Wash the membrane three times in TBST for 5 min each.
5. Incubate the membrane with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h.
6. Wash the membrane three times in TBST for 5 min each.
7. Detect signals using SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo, 34095) capture images with Azure cSeries Capture Software.