

Immunoblot Analysis

1. Total retinal protein is isolated by homogenizing 8 retinas in extraction buffer (PBS [pH 7.4]/1% Triton X-100) and incubating on ice for 1 hr.
2. The total protein equivalent of 0.2 retinas was combined with 4x sample loading buffer and 10X reducing buffer (Invitrogen; Carlsbad, CA).
3. The samples are incubated at 70°C for 10 min and electrophoresed through a 4-12% SDS-PAGE (Invitrogen).
4. The proteins are transferred to PVDF membrane using an X-Cell Blot II module (Invitrogen).
5. The PVDF membranes are blocked in PBS/5% nonfat dry milk/0.1% Tween-20 for 1 hr at room temperature.
6. The membranes are incubated with the primary antibody (diluted in blocking buffer) for 1 hour at room temperature or overnight at 4°C.
7. The membranes are washed three times in PBS/0.1% Tween-20 for 20 minutes at room temperature.
8. The membranes are incubated for 1 hour at room temperature with HRP-conjugated secondary antibody (diluted in blocking buffer).
9. The membranes are washed three times in PBS/0.1% Tween-20 for 20 minutes at room temperature.
10. Densitometry analysis was performed on the X-ray films using the Kodak Image Station 2000r and the Kodak 1D 3.6 software package (New Haven, CT).