Western Blotting

Lysis

1. Lyse tissue in 1x Laemmli buffer (10 x 10^6 cells in 250 μl buffer) by shearing 3x through 20G needle and 5x through 25G needle (or by sonification)
2. Boil lysate 5 minutes at 100˚C.
3. Spin 5 minutes, 13,000 rpm. Store at -20˚C.

Electrophoresis

1. Load 40 μl/slot on normal size SDS stacking gel or 20μl/slot on minigel.
2. Run in 1x Tris-glycin-SDS buffer (Maniatis) for ca. 3 hours or 1 hour respectively at 34mA.
3. Wet transfer or semi-dry blotting onto SS 85BA nitrocellulose membrane.
4. Check transfer by Ponceau Red staining.

Antibody Reaction

1. Block membrane in 10 ml 5% blocking reagent (contained in the Amersham/ECL kit RPN 2108) in PBS-T (PBS+0.1% Tween-20) for >1 hour at RT.
2. Wash 3x 10 minutes with 30ml PBS-T.
3. Incubate filter with 10ml of 1st Ab solution (anti-Enhd1 1:500 in PBS-T) for 1 hour at RT.
4. Wash 5x 10 minutes with 30 ml PBS-T.
5. Incubate with 10ml 2nd antibody solution (ECL kit; use 1:5000 dilution of horseradish peroxidase labeled anti-rabbit Ab in 10ml PBS-T) for 45 minutes at RT.
6. Wash 5x 10 minutes with 30ml PBS-T.
7. Incubate membrane in ECL reagent 1 and 2 (1:1) as indicated by the manufacturer (ca. 1 minute)
8. Dry filter between 2 Whatmann papers. Wrap in Saran wrap and expose for 1 to 15 minutes on film.