

## Western Blotting

1. Prepare Sample in SDS Buffer, BOIL, include ALL controls
2. Run SDS-Page gel, transfer to membrane  
(1X Transfer buffer + 20% Methanol)
3. Prepare TBST: 1XTBS Buffer + .05% Tween20 in 1 liter.  
Prepare 5% blocking buffer (12.5g milk + 250 ml TBST)
4. Incubate membrane 1 hour in Blocking Buffer.
5. Prepare primary antibody dilution in blocking buffer:  
For rabbit antibody of [unknown] - Use 1:1000 Dilution  
For Antibodies of [known] - Use 1  $\mu$ g/ml Dilution  
For activity assay (with high epitope) use 1:2000 anti-myc  
Incubate membrane in 1st antibody + blocking buffer for 1hr.
6. Wash 3X with TBST Buffer 10 minutes each time.  
Wash 1X with Blocking Buffer ~ 20 minutes
7. Prepare secondary antibody dilution in blocking buffer:  
For anti-Rabbit- 1:5000  
For anti-Mouse- 1:4000  
Incubate membrane in 2<sup>nd</sup> antibody + blocking buffer for 1hr.
8. Wash 3X with TBST Buffer 10 minutes each time.
9. Prepare 1:1 Ratio of ECL Solutions from Amersham,  
(2 ml), wet membrane completely with solution, develop