Western Blotting Protocol

Run SDS PAGE gel

Cut membrane to gel size (9cm x 6.5 cm) and soak in transfer buffer

Prepare sponge (4 pieces Whatman blotting paper). Cut to gel size and soak in transfer buffer

Place gel in transfer buffer before transferring

On electrotransfer machine place 2 sponges, the membrane, the gel and 2 more sponges

-role out each sponge layer with a pipet to remove air bubbles

Run at 18V for 20 min

Block the membrane using 5% milk in 1 x TBS-tween for at least 1 hour (or overnight in cold room)

Add primary antibody to 5% milk- TBST in appropriate dilution for at least one hour (or overnight in cold room)

Wash in 1 x TBS-tween 5x's x 5 min or 3x's x 10 min

Add secondary antibody to 5% milk-TBST in appropriate dilution for about 1 hour

Wash in 1 x TBS-tween 5x's x 5 min or 3x's x 10 min

Add membrane to equal amounts ECL solutions 1 and 2 for 1 min

Wrap membrane in saran wrap

Place membrane in cassette and develop with film
**Transfer Buffer (1 liter)**

5.8g Tris base  
2.9g Glycine  
0.37g SDS  
200mL Methanol  
add H₂O to 1L  

**10 x TBST**

100mL of 1M Tris pH 8.0  
20mL of Tween 20  
300mL or 87.66g of 5M NaCl  
Add H₂O to 1L