Western Blot Detection of Protein

1. Obtain from your TA a strip of western blot nitrocellulose membrane. The TA placed the strip into a 15 ml tube and added 5 ml of blocking solution (buffer 1) to the blot.

2. **Start here!** carefully pour off buffer 1, discarding the solution. (Note: all solutions are discarded after they are used, but save the blot!)

3. Add 2 ml (1:5,000) of mouse anti-V5 epitope primary antibody **(1°)** onto the blot and incubate with shaking for 30 minutes. Make sure the blot is laying flat against the tube and is covered with solution. Tape the tube on a shaking platform.

4. Pour off the antibody solution, add 5 ml of buffer 2, shake the blot for 3 minutes. Pour off the wash solution and repeat the washing step one more time. (These steps will wash off unbound antibodies).

5. Pour off the last wash solution, add 2 ml of anti-mouse alkaline phosphate conjugated secondary antibody **(2°)** (1:2500). Incubate at room temperature with the secondary antibody for 15 minutes.

6. Pour off the previous solution and incubate the nitrocellulose filter strip with 5 ml of buffer 2 at room temperature for 3 minutes. Repeat this wash one more time.

7. Pour off the previous solution and incubate the nitrocellulose filter with 5 ml of Buffer 3 at room temperature for 1 minute (this step will equilibrate the filter to the proper pH for hybrid detection).

8. Pour off the solution, add 2 ml of the color detection reagent. Let the color develop under a sheet of foil for 10 minutes to overnight, do not shake the blot. (The length of incubation time depends on the abundance of the protein on the blot).

Buffer 1: (Blocking buffer and antibody binding buffer)

Reagents	Final Concentration
Maleic acid	0.1M
NaCl	0.15M
BSA	1%
Triton X-100	0.3%
pH 7.5	

Buffer 2: (TBS Tween, washing buffer)

Reagents	Final Concentration
Tris -HCl	0.1M
NaCl	0.15M
Tween 20	0.1%
pH 7.5	

Buffer 3: Alkaline phosphatase detection buffer

Reagents	Final Concentration
Tris –HCl	0.1M
NaCl	0.1M
MgCl2	0.05M
pH 9.5	

Color detection reagent

2 ml of buffer 3 with BCIP and NBT (Promega).