Western Blot Protocol**

1. Perform SDS-PAGE on samples to be analyzed.

2. Transfer proteins to Nitrocellulose or PVDF membrane according to the instructions provided by the manufacturer of the transfer apparatus.

3. Stain the blot using Ponceau S* for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in 1xTBS-T* for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk in TBS-T for 1 hour at room temperature up to overnight at 4°C.

6. Dilute the primary antibody in blocking buffer and incubate 1-3 hours at room temperature on a rocking platform.

7. Wash the membrane in TBS-T x3 for 5 minutes and x2 for 8 min on rocking platform and apply the diluted conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.

8. Wash the blot as in step 7 of this protocol.

9. Apply the detection reagent of choice in accordance with the manufacturers instructions.

** The above information is only intended as a guide. The researchers should determine what protocol best meets their needs. Please follow safe laboratory procedures.

* SOLUTIONS:

Ponceau S	10X TBS-T
To make a stock solution, dissolve 0.5g Ponceau S in 100ml of 1% aqueous Acetic Acid. Immerse blot in Ponceau S solution for 5 minutes. Transfer blot to dH2O, and agitate until bands appear (1-5 min). Mark bands (note: bands will fade in 15 min)	166.7 ml of 1.5M Tris-HCl, pH 8.8 73.1g NaCl 5 ml Tween-20 Bring volume to 900 ml with dH2O. Adjust pH to 8.0 Bring final volume to 1000 ml. Store at RT.