Overview

This document explains the materials necessary and the steps to perform a western blot using a secondary antibody.

Additional resources

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* Secondary Antibody
  + Mix 1:1 PBS-Odysssey blocking buffer with 0.2 % tween and 0.01% SDS
    - Eg to make 200mL 199.4 mL of PBS:Blocking buffer and 0.4 mL 100% tween and 200 uL of 10% SDS
  + Wash solution
    - Mix PBS and 0.1% Tween

**Methods**

1. Choose the appropriate secondary antibody for the primary antibody that was used. It should be anti-species used (ex. If primary was mouse, secondary shoul be anti-mouse) and the same class (ex.IgG,IgM,etc.)
2. Prepare 50 mL of solution (BB:PBS + 0.2% Tween+0.01% SDS+antibody) per membrane. Using a 1:7500 dilution for the antibody, you'll need 6.7 uL for 50 mL solution.
3. Incubate on shaker (wrapper in aluminum foil) for 60 minutes.
4. After incubation, wash membrane 4 times for 7 minutes each time on shaker in PBS+ 0.1 % tween (solution C). Keep in dark
5. Rinse the membrane once with PBS. Membrane can remain in PBS until ready to scan (keep in aluminum foil)