Overview

This document explains  the material required, and the steps necessary to perform a western blot-blocking with primary antibody.

Additional resources

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* Blocking Buffer
  + PBS (make PBS solution with 5 tablets in 1 L of DI water)
  + Odyssey blocking buffer
  + Mix 1:1 PBS-Odyssey blocking buffer.
  + Eg:-to make 400 mL mix 200 mL PBS and 200 mL of blocking buffer
* Primary Antibody
  + Mix 1:1 PBS-Odysssey blocking buffer with 0.2 % tween
    - Eg to make 200mL 199.6 mL of PBS:Blocking buffer and 0.4 mL 100% tween
* Wash solution
  + Mix PBS and 0.1% Tween
    - Eg to make 1 L use 999 mL of PBS and 1 mL of 100% Tween

**Methods**

* + 1. Block membrane in a 1:1 Odyssey blocking buffer and PBS solution
    2. Incubate on shaker for 1 hour at room temperature or overnight at 4 C
    3. Choose the primary antibody and make a 1:1000 (15 uL) dilution in 15 mL (per membrane) of 1:1 Odyssey blocking buffer and PBS 0.2 % tween(solution B). For the most part we use 1:1000 dilutions but it can vary, always check
    4. Incubate on shaker for 2 hours at room temperature or overnight at 4 C. This can be cut down to 1 hour at room temperature if needed.
    5. After incubation, wash membrane 4 times for 5 minutes each time on shaker in PBS+ 0.1% tween
    6. \*\*\* All stages beyond this point should be done in the dark (wrapped in foil)