Overview

This document explains  how to perform a western blot with specific antibody.

Additional resources

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* Transfer Buffers
  + - **Anode I (Blotting paper) Add enough deionized water to make 1 L**

|  |  |
| --- | --- |
| **300mM Tris base** | **36.34g Tris** |
| **0.05% SDS** | **2.5 mL 20% SDS** |
| **10% Methanol** | **100mL Methanol** |

* + - **Anode II (PVDF membrane) Add enough deionized water to make 1 L**

|  |  |
| --- | --- |
| **20mM Tris base** | **2.42g Tris** |
| **0.05% SDS** | **2.5 mL 20% SDS** |
| **10% Methanol** | **100mL Methanol** |

* + - **Cathode Buffer (Gel and 2nd blotting paper) Add enough deionized water to make 1 L**

|  |  |
| --- | --- |
| **25mM Tris base** | **3.03 Tris** |
| **0.05% SDS** | **2.5 mL 20% SDS** |
| **40 mM Caproic Acid** | **5.25g Caproic Acid** |
| **10% Methanol** | **100mL Methanol** |

**Methods**

* + 1. Set up 4 containers per gel
    2. Cut membrane to the size of filter and notch one corner being careful not to touch with fingers.
    3. Hydrate membrane in methanol.
    4. Remove the membrane from methanol and place into container marked Anode II buffer. Soak for 15 minutes on shaker
    5. Get out 2 blotting papers and place 1 in Anode 1 buffer and the 2nd in Cathode buffer.
    6. Remove gel cassette from electrophoresis chamber. Crack open both sides. Use spatula to shave off the extra portions of the gel and put a notch in the top left conrer (at about well #1)
    7. Carefully pick up gel and soak gel in Cathode buffer for atleast 5 minutes.
    8. Open the semi-dry transfer cell and stack the contents of the containers in the following order from the bottom to top: 1 blotting paper (Anode 1), membrane (Anode II), gel (cathode buffer), 2nd blotting paper (cathode buffer).
    9. Be sure to align the notches made in the membrane and gel. Use the small roller to roll out any bubbles/creases after putting on the membrane and gel layers to ensure good transfer.
    10. Close lid and set for a constant 0.28 A (0.56 A for 2 gels) for 45 minutes (time will vary according to protein size)
    11. Once complete, gently mark two bands of the ladder on the membrane and label its wavelength with a pencil.