**Western Blot**

**Materials:**

* SDS gel with samples ran but not stained and destained.
* Tris Base
* Glycine
* Methanol
* HCl
* NaCl
* Non-fat Dry Milk
* Ice Block
* Sponges
* PVDF
* Whatman Filter Paper
* Electroblotting Apparatus
* Primary Antibody
* Secondary Antibody
* NBT/BCIP
* Tupperware Container

**Protocol:**

1. Transfer Buffer:

|  |  |
| --- | --- |
| 10x Stock | |
| Tris Base | 30g |
| Glycine | 144g |
| BTV with water | 1L |

BTV: Bring to volume

|  |  |
| --- | --- |
| 1x Transfer Buffer | |
| dH2O | 750ml |
| MeOH | 150ml |
| 10x stock | 100ml |

1. 5x Tris-Buffer Saline (TBS):
   1. Dissolve 30.25g Tris Base in dH2O.
   2. Use concentrated HCl to adjust to final pH 7.4.
   3. Add 22.5g NaCl.
   4. BTV with water 500ml.
2. 1% Blotto Buffer (store in fridge):

|  |  |
| --- | --- |
| Non-fat dry milk | 1.8g |
| 1x TBS | 180ml |

1. 5% Blotto Buffer (store in fridge):

|  |  |
| --- | --- |
| Non-fat dry milk | 2.5g |
| 1x TBS | 50ml |

1. After running SDS-PAGE soak in 1x Transfer Buffer 15-30 minutes.
2. Pre-wet membrane in methanol 10 seconds, and then soak with SDS-PAGE in 1x Transfer Buffer 15-20 minutes.
3. Set up transfer sandwich as follows:

Clear plastic

Sponge

Whatman Filter Paper

Membrane

SDS-PAGE

Whatman Filter Paper

Sponge

Black plastic

1. Transfer 1 hour at 250mAmp with frozen unit at room temperature or overnight in cold room at 30mAmp with frozen unit.
2. Remove membrane and pre-block in 25ml of 5% Blotto Buffer for 30 minutes to one hour at room temperature with gentle rocking.
3. Add primary antibody (ask instructor about it) and incubate 45 minutes at room temperature with gentle rocking, or overnight in cold room with gentle rocking.
4. Wash membrane for 10 minutes three times with 15ml 1% Blotto Buffer at room temperature.
5. Treat with secondary antibody (ask instructor about it) in 25ml of 5% Blotto Buffer for 45 minutes at room temperature.
6. Wash as in step 11.
7. Apply developing reagent (ask instructor about it) until sufficiently developed.
8. Place between two pieces of filter paper and store in lab book to dry.

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