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www.histonecode.com

Western Blot

Revised by Rice Lab
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Procedure

Gel Transfer

We use semi-dry transfer apparatus for transfer.

1. Make "sandwich" of blot paper, membrane, and gel in the following order (bottom to top):
 - 9 pieces of blot paper soaked in 1x Tobin buffer+MeOH
 - Membrane soaked in MeOH
 - Gel soaked in 1x Tobin buffer+MeOH
 - 6 pieces of blot paper soaked in 1x Tobin buffer+MeOH
2. Remove all air bubbles that may be trapped between the pieces of blot paper and to smooth out the gel using a pipet to ensure good transfer.
3. Run transfer for 120 min at 52mA/gel (ex: 2 gels=104mA), place a 1L bottle filled up at least 500mL on top of the apparatus to ensure that the sandwich is held together tightly.
4. Disassemble "sandwich" and place membrane into MeOH for ~5min. To check for good transfer, the gel can be coomassie stained for 1hr and destained for 1 hr after transfer.
5. Stain membrane with Ponceau S to look at transfer quality for 10 min (rocking) and rinse with dH₂O.

Blot

1. Block for 30 min in 5% milk/TBS pH 7.5
2. Seal the membrane in plastic pouch after adding primary antibody (diluted in 1% milk/TBS, 4mL/whole membrane), incubate on Nutator for 1 hr at room temperature. (The incubation time and temperature can be adjusted as the antibody may require)
3. Wash membrane 3x10 min TBS/0.1% Tween-20
4. Seal membrane in plastic pouch after adding secondary antibody (diluted in 1% milk/TBS, 4mL/whole membrane), incubate on Nutator for 1 hr at room temperature. (The incubation time and temperature can be adjusted as the antibody may require)

5. Wash membrane 3x10 min TBS/0.1% Tween-20
6. Develop using ECL kit:
 - 6.1. Dilute 25 μ L of Solution B into 1 mL of Solution A/blot (this solution is light sensitive, so make in a foil wrapped 15mL conical tube)
 - 6.2. Add dropwise to membrane, evenly covering the entire surface—especially around your MW of interest.
 - 6.3. Incubate 5 min, moving foil around to spread out ECL.
 - 6.4. Develop in Room 113B on 1st floor.

It's best to do a 1 min and 3 min exposure to ensure that you are seeing your bands of interest. A shorter or longer exposure may also be required.