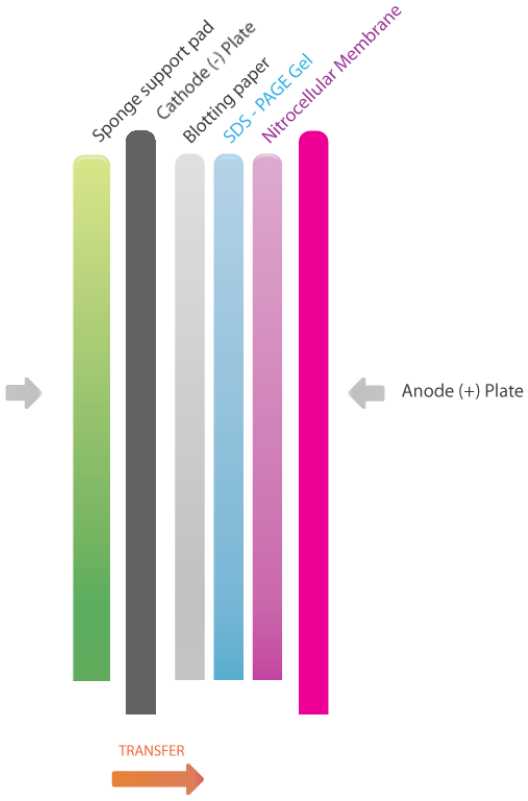
iBlot2 Dry Blotting Transfer

1. Open lid of the iBlot2 device.
2. Using Kit #IB23001 (Thermo Fisher) unseal the Top & Bottom Stack. KEEP THE STACK IN THE PLASTIC TRAY. (One tray can fit two 4-20% Tris-Gly gels)
3. Place the Stack onto the iBlot2 transfer machine making sure the electrical contacts from the stack are aligned with the contact on the left side of the iBlot2 system.
4. Carefully remove the top portion from the stack that contains the Top Cathode portion and place it to the side.
5. Place the pre-run gels onto the transfer membrane making sure that the gel marker is facing toward you and use the Blotting Roller to remove all air bubbles.
6. Place the pre-soaked (in deionized water) iBlot Filter Paper onto the pre-run gels and remove air bubbles using the Blotting Roller.
7. Place the Cathode Stack over the pre-soaked Filter paper with the electrode side facing up and the gel side facing down. Remove air bubbles using the Blotting Roller.
8. Place the Absorbent Pad on top of the Cathode Stack with the metal contact on the left side making sure it aligns with the systems point of contact.
9. Close the lid. Touch P3 on the screen and then start and it will run for 7 minutes.
10. Once the iBlot2 beeps indicating that the cycle is complete, open the lid of the iBlot2 device.
11. Discard the Disposable Sponge and Cathode Stack .
12. Carefully remove and discard the filter paper. Use gels as a marker and cut the transfer membrane in half using a razor.
13. Discard gels and remove the transfer membrane from the stack. Discard the Anode stack after you removed the membranes for testing.
14. Incubate the membrane in blocking solution (10% Seablock for all antibodies) for 40 minutes with gentle agitation on an orbital shaker.
15. Wash the membrane three times with TBST (TBS, pH 7.2 with 0.1% TWEEN-20) in a clean tray on an orbital shaker; each wash lasting 5-10 minutes.
16. Dilute the probing (primary) antibody in TBST to a volume of 50ml (approxi mate final concentration of 0.20µg/ml) and incubate the membrane in the anti body solution for one to four hours at room temperature. (The optimum incuba- tion time depends on the antibody/antigen binding affinity and must be pre-de termined for each antibody.)
17. Wash the membrane three times as in step #15.



iBlot2 Dry Blotting Transfer continued....

18. Dilute the secondary antibody in TBST according to the manufacturer's specification. Incubate the membrane in a clean tray containing 50ml of diluted secondary antibody for one hour at room temperature on an orbital shaker.

19. Wash the membrane three times as in step #15.

20. Color development requires the use of a commercially available (e.g. Bio-Rad or Sigma) alkaline phosphatase conjugate substrate kit. Follow the manufacturer's instructions.

21. After the bands become clearly visible, stop the color by placing the membrane in a tray containing diH2O for at least ten minutes.

Recommended Materials:

- SDS-PAGE apparatus and accessories
- iBlot2 Dry Blotting Transfer System
- iBlot2 NC Regular Stacks
- Seablock
- 1xTBST
- Antigen specific probing antibody (Primary Antibody)
- Secondary Antibody (Donkey-anti-probing antibody species conjugated to alkaline phosphatase)
- Commercially available alkaline phosphatase conjugate substrate kit