PVDF Membrane Blotting Procedure

Solutions:

100mM CAPS:	Transfer Buffer:
22.1g CAPS (Sigma Cat# C-2632)	10% Methanol (HPLC grade)
5g NaOH	10% 100mM CAPS Buffer
950ml water	80% water
Adjust pH to 11with NaOH	

Staining Solution:	Destaining Solution:
0.1% Amido Black*	5% Acetic Acid
10% Acetic Acid	

*Coomassie and Colloidal Coomassie stains also acceptable.

Materials:

Membrane from Millipore: Immobilon P (SQ) PVDF membrane.
(DO NOT USE NITROCELLULOSE)
At least 15pmol total protein on SDS-PAGE gel transferred onto membrane.

Procedure:

1. After electrophoresis of sample, soak gel in transfer buffer for 5-10 mins.
2. Prewet PVDF SQ membrane with 100% methanol (HPLC grade), then soak membrane in transfer buffer for 5-10 mins.
3. Assemble Blot apparatus:
    a. (-) Plastic plate
        2 pieces Whatman filter paper
        Gel
        PVDF membrane
        2 pieces Whatman filter paper
    (+) Plastic plate

Roll out air bubbles with a plastic pipette between each step.

4. Blot for 1-3 hrs at 70-80 volts (0.35-0.4) at 4^{o}C with stirring of buffer. (Length of time depends on Mwt of protein. Larger proteins may require transferring overnight at 40 volts in the cold).
5. After blotting, stain with amido black (or similar) for 1-2 mins.
6. Destain with 3-4 changes of destain solution. Background staining can be reduced by rinsing the blot with 100% methanol (HPLC grade).
7. Bands can be cut from the wet membrane and stored at -20 ^{o}C.