

ELECTROTRANSFER OF PROTEINS TO PVDF MEMBRANE FOR SEQUENCE ANALYSIS

This protocol is preferred for proteins destined to automated Edman sequencing and is amenable to all other downstream cleavage methods. The use of CAPS buffer is important in order to eliminate the high concentrations of glycine that result from Towbin buffer that will interfere with the ability to accurately determine the protein sequence of the protein. The method can be used for electroblotting for Western analysis, but is more costly due to the higher cost of the CAPS.

REQUIRED SOLUTIONS

Electroblot Buffer: 10 mM CAPS
10% methanol
pH 11.0

10x Concentrated Stock

Measure ~900 ml MilliQ water
Add 22.13 gm CAPS
Titrate to pH 11.0 with concentrated NaOH
Bring to 1000 ml with additional water.

1X Working Solution

800 ml Milli-Q water
Add 100 ml HPLC grade MeOH
Add 100 ml 10x concentrated stock of CAPS

PROCEDURE (wear gloves for all procedures)

1. Cut 10 x 10 cm square of PVDF membrane on a clean glass with a sharp and clean razor blade.
2. Prewet cut PVDF membrane with Methanol.
3. Soak membrane in transfer buffer for 5-15 minutes.
4. Soak gel in transfer buffer for 15-30 minutes.
5. Prepare a sandwich of soaked gel and PVDF between Whatman 3MM (or similar) paper and assemble the blotting cassette according to the list below.

IT IS CRITICALLY IMPORTANT TO NOT CAPTURE AIR BUBBLES BETWEEN THE MEMBRANE AND THE GEL. THIS WILL BLOCK TRANSFER OF PROTEINS TO THE MEMBRANE.

6. Lay down a cassette with Black side down and one sponge in a Tupperware; flood the cassette with some transfer buffer and rub the air bubbles out of the sponge.
7. Lay a piece of wet 3MM paper on the sponge.
8. Lay the soaked gel on the paper; Smooth out bubbles.
9. Lay the soaked PVDF membrane on the gel; Smooth out bubbles.
10. Lay a piece of wet 3MM paper on the membrane.
11. Lay a wet sponge on the paper.
12. Close and clip the cassette.
13. Transfer the cassette to the blotting tank prefilled with transfer buffer.

You should have a layer of

SPONGE
BLOTTER PAPER
GEL
PVDF MEMBRANE
BLOTTER PAPER
SPONGE

14. Electrotransfer the proteins TOWARDS THE ANODE FOR 1 HOUR AT 100 mA.

Note: These conditions are for a 0.75 mm thick gel for proteins in the range of 25-65 kDa. Larger or lower molecular weight proteins usually require longer transfer times. Other tricks include including some SDS in the transfer buffer or lowering the MeOH concentration.

You will need longer times for thicker or higher concentration gels. For example, 1.0 mm thick gels (6-10%) or 12-15% gels, use 1.5-2 hours at 100 mA.

15. Disassemble the cassette and stain the PVDF membrane with Amido Black for one minute in Tupperware container. **DO NOT ALLOW MEMBRANE TO DRY OUT.**
16. Destain the PVDF membrane in 1% acetic acid with repeated changes until the bands are clearly visible and the background is reduced.
17. Wash the membrane in MilliQ water for several minutes before air drying between two sheets of 3MM paper.
18. Stain the gel with Coomassie Blue R-250 for one hour. (optional)
19. Destain the gel with Coomassie destain. (optional)

ALTERNATIVE STAINS.

- **Coomassie blue R-250:** 0.1% Coomassie blue in 50% methanol/1% acetic acid, 5 min. Destain: 1-3 times with 50% methanol/1% acetic acid (5 min) followed by several washes with MilliQ water.
- **Coomassie blue G-250:** Stain for one to several minutes. Destain with water. (More sensitive than Amido Black, but 'dirtier'. Causes higher sequencer background noise).
- **Ponceau S:** 0.1% Ponceau S in 1% acetic acid, 5 min. Destain: 1% acetic acid, followed by several MilliQ water washes. (Least sensitive but very clean stain. Low sequencer noise)