SOP FOR ELECTROTRANSFER OF SDS-POLYACRYLAMIDE GELS

TRANSFER BUFFER

CAPS buffer is preferred for N-terminal sequence analysis because the background is significantly lower and there is no glycine present to contaminate the first cycle of automated Edman degradation.

CAPS: 10 mM CAPS, 10% methanol, pH 11.0. Degas before use.
(CAPS = 3-[cyclohexylamino]-1-propane-sulfonic acid); Sigma C-6070)

To prepare 1 liter of a 10X concentrated stock solution:

- ❑ Place 900 ml Milli-Q water in a 1 liter beaker; insert stir bar
- ❑ Dissolve 22.13 gm CAPS (chemical shelf, RT) in water
- ❑ Titrate dropwise with 10 M NaOH to final pH
- ❑ Transfer to a 1000 ml graduated cylinder
- ❑ Add Milli-Q water to 1000 ml
- ❑ Store at 4C

To prepare 1 liter of working solution:

- ❑ Measure 800 ml of Milli-Q water into a 1000 ml graduated cylinder
- ❑ Add 100 ml of 10X stock CAPS buffer
- ❑ Add 100 ml 100% HPLC grade methanol
- ❑ Mix well
- ❑ Store at RT

GEL AND MEMBRANE EQUILIBRATION

❑ Cut PVDF membrane to the same size as the gel. Pre-wet the PVDF by immersion in 100% methanol for 30 sec, followed by equilibration in transfer buffer for 5-15 min.

❑ Equilibrate the gel in transfer for 15-30 min (depending upon gel thickness).

This is actually an important step. Many scientists do not equilibrate their gel. This leads to a drastic reduction in blotting efficiency.

BLOTTING

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

MATERIALS:

- ❑ One Large staining tray
- ❑ One Small staining tray with CAPS buffer
- ❑ Equilibrated PVDF membrane
- ❑ Equilibrated gel
- ❑ 10 x 10 cm gel blot paper-2 pieces
- ❑ Mini-gel blotting sandwich cassette materials (frame, 2 sponges)
- ❑ 1X CAPS buffer

PROCEDURE:

- ❑ Pour a small amount of CAPS buffer into a small staining tray
- ❑ Place one sponge in tray
- ❑ Place two sheets of gel blot paper in tray
- ❑ Place another sponge in tray
- ❑ Make certain that the transfer tank has CAPS buffer
- ❑ Place the blotting sandwich frame into the large staining gray with the **black side down**
- ❑ Place one sponge on the frame
- ❑ Pour enough CAPS buffer into the tray to just cover the frame and sponge
- ❑ Place a piece of soaked gel blot paper on the sponge
- ❑ Place gel on paper
- ❑ Place equilibrated PVDF membrane on the gel
- ❑ Place another piece of soaked gel blot paper on the membrane
- ❑ Place a soaked sponge on the paper
- ❑ Close and snap shut the sandwich cassette
- ❑ Transfer immediately to the transfer tank

Transfer settings: **ALL GELS TRANSFERRED AT 100 mamps (constant)**

10% acrylamide, 0.75 mm spacer	1 hour
15% acrylamide, 0.75 mm spacer	1.5 hour
10-15% acrylamide, 1 mm spacer	2 hours
10-15% acrylamide, 1.5 mm spacer	3 hours