

Electroblotting Procedure

Electrophoresis

The electrophoresis apparatus can be of any manufacturer. The gel apparatus used in the BSI is manufactured by Bio Rad. The electrophoresis is carried out using the method of Laemmli(Nature:277,680,1970). Several other gel systems have been used with no problem.

Electroblotting

The electroblotting apparatus that have been used in the facility are all Bio Rad tank and semidry units.

Electroblotting Membranes

Membranes from many manufacturers have been evaluated by transfer of known proteins in quantitated samples. The following membranes have given satisfactory N-terminal sequencing and internal sequencing results.

Product	Source	Part#
Problott	Applied Biosystems Inc.	400994
Immobilon PSQ	Millipore Corp.	ISEQ20200
PVDF	Bio Rad	162-0182

Chemicals

Product	Source	Part#
Methanol	Burdick and Jackson	230-4
Acetonitrile	Burdick and Jackson	015-4
Trifluoroacetic Acid	Pierce Chemical Co,	28903
CAPS (3-[cyclohexylamino]-1-propane-sulfonic acid	Sigma	C-2632

Ponceau S	Sigma	P-7767
Coomassie R-250	Sigma	B-0630
Bromophenol blue	Bio Rad	161-0404
Acetone	J.T. Baker	9017-02
Tween 20	Aldrich	27,434-8
Ammonium persulfate	Bio Rad	161-0700
Glycine	Bio Rad	161-0717
N,N'-methylene bis-acrylamide	Bio Rad	161-0200
N,N,N,N,-tetra-methyleneamine	Bio Rad	161-0800
Acrylamide	Bio Rad	161-0100
Sodium Bicarbonate	J.T. Baker	3003-01
2-mercaptoethanol	Sigma	M-6250
N-ethylmorpholine	Pierce	20805
Glycerol	Fisher	BP229-1
Acetic Acid	J.T. Baker	9508-04
Amido Black	Sigma	A-8181

Electroblotting Method

The transfer is performed by the method of Matsudaira(J.Biol.Chem.;262,10035,1987).

- CAPS working solution:
 - Stock Solution 10X
 - 22.13g of CAPS in D.I. water 800 mL
 - pH to 11.0 with NaOH
 - adjust volume to 1000 mL
- Working Solution 1X
 - 200 mL of Stock 10X CAPS added to 200 mL MeOH and 1600 mL D.I. water
- Cut PVDF membrane to the size of the gel

- Wet membrane with methanol for a few seconds.
- Soak membrane in blotting buffer for five minutes
- Soak gel for five minutes in blotting buffer
- Soak pads and filters in blotting buffer and assemble the transblott unit
- Electroblott at 50 volts constant voltage for 20 minutes to one hour (for proteins from 80-100,000 and larger delete the methanol)
- Remove the membrane and the gel from the sandwich rinse with D.I. water

Staining

All conventional methods of staining may be employed with PVDF membranes with slight modifications as below.

- Coomassie R-250:
 - Soak blott(after D.I. water rinse) in methanol
 - Stain PVDF membrane with 0.1% Coomassie R-250 in 40% MeOH for no longer than ONE MINUTE usually 15 to 20 seconds will suffice. (Staining for longer periods of time will result in high background and will interfere with extraction and cleavage)
 - Destain with 50% methanol, several changes
 - Rinse extensively with D.I. water
 - Cut out the band of interest
- Ponceau S:
 - Stain the PVDF membrane in 0.25% Ponceau S in 1% Acetic acid for one to three minutes(until protein bands are visible)
 - Destain in D.I. water
 - Excise the band of interest
 - Rinse with D.I. water
- Amido Black:
 - Soak blott after D.I. water rinse with methanol
 - Stain PVDF membrane with 0.2% Amido Black in 40% MeOH for 30 seconds to one minute
 - Destain in D.I. water with multiple changes until bands are clear and low background
 - Cut out band of interest