IMMUNOBLOTTING PROTOCOLS

Reference:
- Immobilon-P transfer membrane user guide. Millipore
- Instructions for Supersignal® Elisa Femto maximum Sensitivity Substrate. Pierce

Immobilon-P membranes from Millipore (catalogue # IPVH07850) works well for both immunoblotting protocols.

Alkaline Phosphatase Immunoblotting

SOLUTIONS:

Tris-Buffered Saline (TBS pH 7.5)

- 50 ml 1M Tris.HCl pH7.5
- 40 ml 5M NaCl
- 910 ml dH2O
- 1L total volume

Tris-Buffered Saline plus 0.05% (v/v) Tween 20 (TBST pH 7.5)

- Dissolve 0.5 ml Tween 20 in 1L TBS

BBS plus 0.1% Tween 20 (BBST)

- 6.18 gm Boric acid
- 9.5 gm Sodium borate
- 58.4 gm Sodium chloride
- 1ml Tween 20
- in 1L total volume

Substrate Buffer

- prepare fresh
- 3.5 ml 1M Tris. HCl pH9.5
- 0.7 ml 5M NaCl
- 1.75 ml 1M MgCl2
- 29.05 ml dH2O
- 35 ml total volume

BCIP and NBT in Substrate Buffer

- 35 ml substrate buffer
- 154 µl NBT
- 116 µl BCIP

Phosphate Buffered Saline (PBS)

- 2.135 gm K2HPO4
- 1.055 gm KH2PO4
- 16.36 gm NaCl
- Make up to 2L with water
- pH should be 7.0-7.2
- I usually prepare a 10 x stock solution
PROTOCOL

1) Block in TBST pH 7.5 + 5% (w/v) nonfat dry milk, 1-2 hours at room temperature with agitation.

2) Incubate with 1° antibody in TBST pH 7.5, overnight at room temperature with agitation.
   I use:
   - 1:7000 dilution of 12CA5 Ab lot # SCP-12CA5-E purchased from Berkeley Antibody Co.
   Inc.
   - 1:5 dilution of 9E10 Ab obtained from the Hybridoma Facility, University of Wisconsin
   biotechnology Center, Madison, WI

3) Wash - 2 x for 10 minutes in TBST pH 7.5
   2 x for 10 minutes in BBST
   2 x for 10 minutes in TBST pH 7.5

4) Incubate with 2° antibody in TBST pH 7.5, 1 hour at room temperature with agitation.
   I use a 1:5000 dilution of goat anti-mouse IgG antibody-alkaline phosphatase (calf
   intestine) conjugate purchased from Boehringer mannheim.

5) Wash - 1 x briefly in TBS pH 7.5
   2 x for 15 minutes in TBS pH 7.5

6) Incubate with BCIP and NBT (purchased from Gibco BRL) in substrate buffer, for a
   maximum of 30 minutes.

7) To stop reaction, rinse with PBS containing 20mM EDTA.

8) Air Dry in the dark.
ECL Immunoblotting

With the ECL Western blotting system from Amersham

SOLUTIONS:

Tris-Buffered Saline plus 0.05% (v/v) Tween 20 (TBST pH 7.5)
50 ml 1M Tris.HCl pH7.5
40 ml 5M NaCl
0.5 ml Tween 20
910 ml dH2O
1L total volume

Tris-Buffered Saline (TBS pH 9.5)
50 ml 1M Tris.HCl pH9.5
30 ml 5M NaCl
920 ml dH2O
1L total volume

Tris-Buffered Saline plus 0.5% (v/v) Tween 20 (TBST pH 9.5)
50 ml 1M Tris.HCl pH9.5
30 ml 5M NaCl
5 ml Tween 20
915 ml dH2O
1L total volume
1) Block in TBST pH 7.5 + 5% (w/v) nonfat dry milk, 1-2 hours at room temperature with agitation in a heat sealed bag.

2) Incubate with 1° antibody in TBST pH 7.5, for one hour to overnight at room temperature with agitation in a heat sealed bag.
   I use:
   - 1:7000 dilution of 12CA5 Ab lot # SCP-12CA5-E purchased from the Berkeley Antibody Co. Inc.
   - 1:5 dilution of 9E10 Ab obtained from the Hybridoma Facility, University of Wisconsin biotechnology Center, Madison, WI

3) Wash -rinse 2 x in TBST (pH 7.5)
   1 x for 15 minutes in TBS (pH 9.5)
   2 x for 5 minutes in TBS (pH 9.5)

4) Incubate with 2° antibody in TBST (pH 9.5) for 1 hour at room temperature with agitation.
   I use a 1:40,000 dilution of the Mouse Ig, horseradish peroxidase-linked whole antibody (from sheep) supplied with the ECL Western blotting analysis system for Amersham (cat # RPN 2108).

5) Wash -rinse 2 x in TBST (pH 9.5)
   1 x for 15 minutes in TBS (pH 9.5)
   3 x for 5 minutes in TBS (pH 9.5)
   1 x for 5 minutes in TBST (pH 7.5)

6) Detection - follow instructions for the ECL Western blotting analysis system or for the SuperSignal® Elisa Femto substrate. I use Hyperfilm ECL from Amersham or the maximum sensitivity substrate from Pierce.