

IMMUNOBLOTTING PROTOCOLS

Reference:

- Immobilon-P transfer membrane user guide. Millipore
- Atkin, A.L., N. Altamura, P. Leeds, and M.R. Culbertson. 1995. The majority of yeast UPF1 co-localizes with polyribosomes in the cytoplasm. Mol. Biol. Cell 6: 611-25
- 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

PROTOCOL

- 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^o 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^o 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.