General Immunoblotting Protocol

Immunoblotting is typically used to determine the amount (dot blot) and molecular weight (Western blot) of an antigen present in a complex mixture. The highly sensitive procedure below is suggested. All incubations are at room temperature. This procedure is for nitrocellulose or PVDF membranes. Do not to touch the membrane! Wear gloves. PVDF membranes must be pre-wet with 100% methanol before equilibrating in transfer buffer. Nylon membranes may be used; however, they are more difficult to block. To block nylon membranes, use buffer without Tween-20, replace BSA with 10% non-fat dry milk and block for several hours to overnight at 4°C. When selecting membranes, the specifications of the particular imaging systems with their available filters must be taken into consideration. Some membranes may exhibit autofluorescence at certain wavelengths.

I. Reagents Required

Reagent	Preparation
Tris Buffered Saline with Tween-20 (MB-013)	Use 10X TTBS, pH 7.5 (1.0 M Tris HCl, 1.5 M NaCl, 0.1% Tween-20). Dilute appropriate volume to 1X with deionized water. Store at room temperature up to one month.
TTBS with 1% BSA	To 100 mL of 1X TTBS, add 1.0 g of Bovine Serum Albumin (BSA)(BSA-10). Dissolve and use immediately. Our Blocking Buffer for fluorescent Western blotting (MB-070) is also suggested.

II. Procedure

1. Transfer and immobilize antigen on nitrocellulose or PVDF membrane.
2. Block for at least 30 minutes with gentle agitation by immersing the membrane in TTBS or TTBS 1% BSA. Use just enough solution to cover the membrane. Never let the membrane become dry during the procedure. 1.0% BSA to blocking solution increases signal-to-noise ratio over the use of TTBS alone. Our Blocking buffer for fluorescent Western Blotting (MB-070) is also suggested. Some antigens and antibodies may be eluted in the presence of Tween-20. If this occurs, replace the Tween-20 with 1.0% BSA in all TTBS solutions and repeat the experiment.
3. Transfer the membrane to diluted solution of primary antibody in TTBS or blocking buffer for fluorescent Western blotting (MB-070). The appropriate dilution should be determined by trial and error. Serial tenfold dilutions starting at 1:10 are suggested. Incubate for 30 minutes to 2 hours with gentle agitation.
4. Wash with 3 changes of TTBS for 5 minutes each with gentle agitation.
5. Transfer the membrane to a dilute solution of DyLight™ or other fluorescent-dye conjugated secondary antibody in suitable buffer such as or a 1.0 % BSA in TTBS, blocking buffer for fluorescent Western blotting (MB-070) or TTBS. Confirm reagent specificity for primary antibody. DyLight™ or any fluorescent-dye conjugated antibody should have exposure to minimized light. Use opaque incubation box or wrap in foil. Incubate for 30–45 minutes with gentle agitation.
6. Repeat step 4 (Minimize exposure to light).
7. Rinse membrane in DI H_2O, allow to dry with minimized exposure to light.
8. Image at appropriate wavelength for particular DyLight™ Conjugate. Consult product certificate of analysis for absorption andemission wavelengths and imaging system instructions for appropriate filters.

References

- Antibodies, A Laboratory Manual. Ed Harlow and David Lane, eds. Cold Spring Harbor Press, 1988. Chapter 12 gives an excellent overview of Western blotting techniques, including India Ink staining.
- Molecular Cloning: A Laboratory Manual. 2nd Edition. J. Sambrook, E.F. Fritsch and T. Maniatis, eds. Cold Spring Harbor Press, 1989. Chapter 18 gives detailed protocols for the production of cell lysates, electrophoresis and blotting of proteins.
- Antibodies, A Practical Approach. 2nd Edition. Catty, D., ed. IRL Press, Oxford, England, 1990. Volumes I and II represent a detailed and complete reference for most current antibody techniques