



IMMUNOBLOTTING (WB) - PROTOCOL

Principle

Immuno-blotting is typically used to determine the amount (dot blot) and molecular weight (western blot) of an antigen present in a complex mixture. Assay sensitivity can be modulated by using different enzyme substrates and/or by using biotin-(strept)avidin conjugates in place of an enzyme conjugated secondary antibody. The highly sensitive procedure is listed below. If decreased sensitivity is desired, replace the biotin-streptavidin conjugate with an antibody-peroxidase conjugate. All incubations are carried at room temperature.

Reagents Required

- Tris Buffered Saline with Tween-20.
Use 10x TTBS, pH 7.5 (1.0M Tris HCl, 1.5M NaCl, 0.1% Tween-20). Dilute appropriate volume to 1x with de-ionized water. Store at room temperature up to one month only.
- TTBS with 1% BSA
Add 1.0g of Bovine serum albumin (BSA) to 100ml of 1x TTBS. Dissolve and use immediately.

Procedure

1. Transfer and immobilize antigen on nitrocellulose or PVDF membrane.
2. Block by immersing the membrane in TTBS. Use just enough solution to cover the membrane. Never let the membrane become dry during the procedure. Incubate for 30 minutes with gentle agitation. The addition of 1.0% BSA to the blocking solution increases signal-to-noise ration over the use of TTBS aloe. 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. The appropriate dilution should be determined by trial and error. Serial ten fold dilutions starting at 1:10 are suggested. Incubate for 30 minutes 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 biotinylated secondary antibody in TTBS. Confirm reagent specificity for primary antibody. Incubate for 30 minutes with gentle agitation.
6. Repeat step 4.
7. Transfer the membrane to a solution of peroxidase conjugated streptavidin appropriately diluted in TTBS. Incubate at 30 minutes with gentle agitation.

- 8.** During the above incubation, prepare the enzyme substrate. Use DAB for a dark brown color or TMB for a bright blue color.
- 9.** Wash as in step 3.
- 10.** Transfer the membrane to the substrate solution. Incubate until color develops sufficiently (usually 2-20 minutes).
- 11.** Wash with 2 changes of water for 5 minutes each with gentle agitation. Allow membrane to dry and store in the dark.