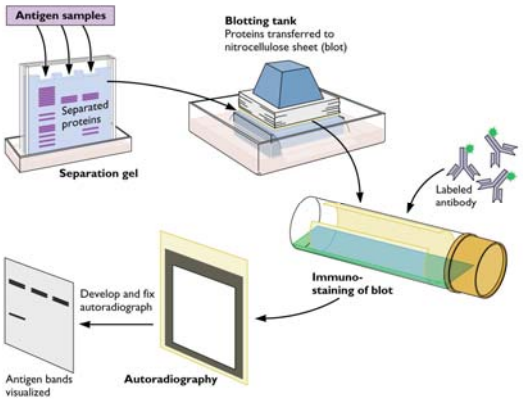
Quick Protocols

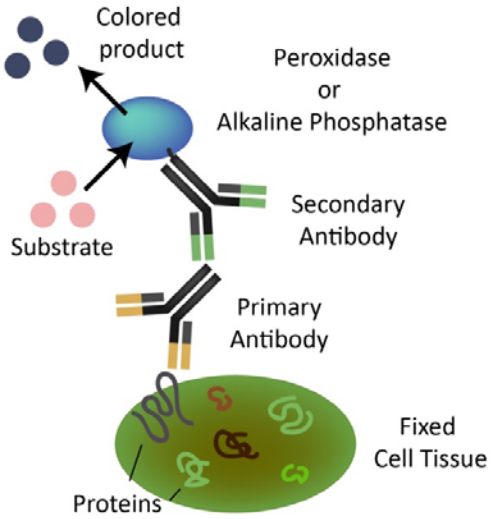
Western Blotting



1. Run the gel
2. Transfer proteins from gel to membrane
3. 90 minutes at 1mA per cm^2 should do it
4. Block the membrane for an hour
5. Add primary antibody, incubate and then wash
6. Add secondary antibody, incubate and then wash
7. Develop with ECL and marvel at your results

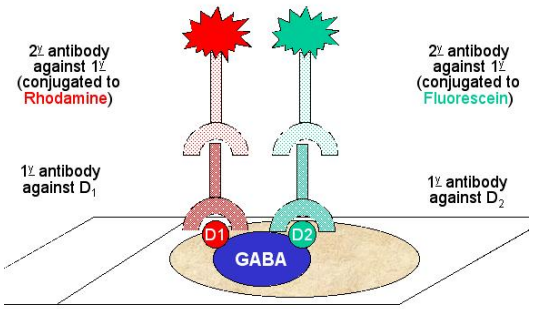
Immunohistochemistry

1. De-paraffinize slides in two changes of xylene
2. Incubate in 100% alcohol, 95%, 80%, 60% and then water for 5 min each
3. Place slides in antigen retrieval buffer, microwave for several min and then wash in TBS
4. Incubate in 3% H_2O_2 in methanol for 30 min and then wash in TBS
5. Block with 5-10% goat serum for 30 min
6. Add primary antibody, incubate and then wash
7. Add biotinylated secondary antibody, incubate and then wash
8. Apply streptavidin peroxidase for 15 min and then wash
9. Develop with chromogen before rinsing with running tap water
10. Counterstain with Mayer's hematoxylin for 30-60s before extensive washing
11. Dehydrate through 60%, 80%, 95% and 100% alcohol for 5 min each
12. Transfer to xylene for 5 min beforemounting and results



Immunofluorescence

1. Fix cells with formalin-PBS (10%) for 10 min at 4°C
2. Place in ice-cold acetone (-20°C) for 5 min
3. Transfer the cover glass into PBS before blocking with BSA-PBS-NaN3
4. Add primary antibody (1μg/ml) and incubate for 1 hr at RT in the dark
5. Wash extensively with PBS-NaN3
6. Add fluorescently-labeled secondary antibody and incubate for 1 h at RT in the dark
7. Mount cover slip in 15% glycerol and seal the edges with nail polish



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