LR White for Immunohistochemistry

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Using LR White for Immunohistochemistry

Sections from LR White embedding tissue have been used successfully for immunocytochemistry at both the light microscope and electron microscope levels. This demonstrates quite clearly that the visualization steps of the immunocytochemical procedure will penetrate the resin and react with tissue antigens if they have been preserved in tissue.

As with all immuno-localizations the key factor is whether or not the tissue antigen has survived fixation, processing and embedding in such a form as to be recognizable to the specific antibody. This is difficult to predict with certainty, but some antigens have been shown to be highly resistant whilst others are fickle even in unfixed frozen sections.

Much interest has centered on using immunocytochemistry to detect protein hormones and the various classes of immunoglobulin and generally these classes of antigen have proved resistant to alteration both in processing to paraffin wax and to LR White resin.

It is the special hydrophilic nature of LR white which allows immunochemicals to permeate the supporting resin and reach its sites of binding and no resin pretreatment is necessary, or indeed possible, to facilitate this penetration.

We have been successful with LR White blocks only when they have been thermally cured, probably because when accelerator-curing the resin the exotherm produced is sufficient to damage the integrity of the tissue antigen. Some workers have also reported that a slight under-cure of LR white say at 55° C for 20-24 hours aids subsequent penetration of antisera, but we have obtained good results without deviating from the standard polymerization schedule.

If the particular antigen under consideration has already been localized in paraffin wax sections then a trusted fixation regime will be established and should be adhered to. For those approaching the problem for the first time there is an extensive bibliography available regarding fixation for immunocytochemistry, much of it contradictory, and a reference list is provided as some guidance. Rules of thumb seem to be to avoid Glutaraldehyde and perhaps use an acid rather than a neutral fixative, but there are many conflicting and strongly held views on the topic.

Similarly, the need to enzyme digest sections prior to reaction is fraught with controversy and may indeed be linked to the fixation regime chosen. We have used both protease type VII and trypsin type II to good effect on our neutral buffered Formalin fixed materials.

If frozen, dewaxed or etched epoxy resin sections are used for immuno-staining, the tissue is not surrounded by a supporting matrix when they are being reacted. When using LR White sections the resin is still intact and therefore diffusion to the sites of reaction must occur prior to reaction of antisera with antigen. For this reason we have found it necessary to use antisera at approximately ten times the concentration that would work on dewaxed sections. The exact titre of each antibody will depend upon its source and how well it has been stored, but we have used many commercial anti-immunoglobulins at about a 1 in 10 dilution.

For the same reason the antibody stages of the reaction often benefit from a longer incubation time. Up to two hours at room temperature or overnight at 4° C in a moist chamber may be used.

Various immunoperoxidase techniques have given results on LR White tissue sections including the peroxidase-antiperoxidase complex method (PAP) (Sternberger, 1970), the hapten sandwich technique (Jasani et al, 1981), and the indirect peroxidase method. The Avidin-biotin-peroxidase complex method if Hsu has not been successful in our hands with LR White embedding material, probably due to the molecular size of Avidin. As fairly strong antibody concentrations are required, a highly sensitive method of detection is to be preferred and for this reason the PAP or hapten sandwich techniques are more suitable than a two-layer indirect peroxidase reaction.

Visualization for the bound peroxidase is achieved with the diaminobenzidine–peroxide reaction as described by Graham & Karnovsky (1966).

Any technique where the sections are subjected to hydrogen peroxide solutions twice during staining is likely to tend to lift sections from the slides. We have found that poly L lysine (MW 350,000) is an excellent adhesive for immunocytochemical work, and also care should be taken to dry sections onto slides very thoroughly in an oven rather than a hot plate at 60° C for two hours. This step should not have any effect on the antigenicity of the tissue, as it will already have spent 20–24 hours at 60° during polymerization.

It is clear that not "standard" immunohistochemical staining regime can be cited as there are some any variables, but a "typical" regime is described below for general guidance.

Pap Procedure for L.R. White Sections (3 micron)

1. Block EndogenousPeroxidase with 1% Phenylhydrazine Hydrochloride in PBS (optional) .. 30 minutes
2. Wash in PBS 1x5 minutes, and 2x5 minutes at 37° C
3. 0.1% Trypson in 0.1% CaCl2 (aqueous) ··· 20 minutes
4. Wash in ice cold distilled water ··· 10 minutes
5. 2% Goat serum in PBS··· 20 minutes
6. 1° antibody (approx. 1:10 dilution) 2 hours at 37° C or overnight at 4° C (usually Dako or Nordic)
7. Wash in P.B.S···10 minutes
8. Goat anti rabbit antibody (approx. 1:20 dilution) 2 hours at 37° C or overnight at 4° C.
9. Wash in P.B.S. ··· 10 minutes
10. PAP at 1:10 dilution (Dako) 2 hours at 37° C or overnight at 4° C
11. Wash in P.B.S. ··· 10 minutes
12. Wash in Tris HCl pH 7.6 ··· 10 minutes
13. DAB/H2O2 (Graham and Karnovsky) ··· 15 minutes
14. Wash in distilled water.
15. Counterstain as required.

Note: FOR IMMUNOCYTOCHEMISTRY LR WHITE MUST BE THERMALLY CURED AND NOT ACCELERATOR CURED.

References

Curran, R.C. and Gregory, J., The unmasking of antigens in paraffin sections by Trypsin. Experientia, 33; 1400 (1977).

Elias J.M., Principles and Techniques in Diagnostic Histopathology. Published by Noyes Publications New Jersey, USA (1982) ISBN – 0-8155-0903-0.

Graham R.C., and Karnovsky, M.J., The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J. Histochem. Cytoche, 14, 291 (1966).

Jasini, B., Wynford-Thomas, D., and Williams, E.D., Use of monoclonal anti-hapten antibodies for immunolocalizations of tissue antigens. J. Clin. Path. 34, 1000 (1981).

Newman, G.R., Jasanai, B., and Williams, E.D., The preservation of ultrastructure and antigenicity, J. Microscopy, 127, RP5-RP6 (1982).

Sternberger, L.A., Hardy, P.H., Jr., Cuculis, J.J. and Meyer, H.G., The unlabelled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody comples (horseradish peroxidase-antihosreradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18, 315 (1970).