Immuno-EM

Immunocytochemistry for TEM

Unfortunately it is rare that any two experiments employing immunocytochemistry are alike. There are many factors which will govern the course and specific details of an experiment. Trial and error play a large role in the eventual outcome.
Literature Review

The starting point for any immunocytochemistry experiment, however, is to review the literature to see if someone else has developed procedures which preserve morphology and labeling of epitopes, so establishing a credible starting point for your experiments. Remember what is published will be a summary of the procedure used and may not contain all necessary information to repeat the process and may also not contain all the trials and tribulations involved in getting a publishable result. If no one has ever succeeded with your tissue, you will probably face more challenges.

Immuno-fluorescence tests

As with most localization procedures, it is probably better to try at the light microscope level first. It is possible to get a rough idea of antigen localization quite quickly (few hours) and antibody specificity. Controls should be employed to establish the validity of the localization using similar methods as proposed for the later electron microscopy. Many conditions (fixation, etc.) that optimize localization can be determined with light microscopy.

Primary Antibody

It is important to obtain a primary antibody directed against the specific antigen to be localized. Everything hinges on the quality of the primary antibody. Antibodies with poor specificity or poor affinity will give poor results. Many primary antibodies are available commercially, however, they are usually expensive and provided in small quantities. It may be necessary to obtain antibodies from another investigator or prepare them from scratch. It is important that the primary antibody preparation should be biochemically clean. Even though commercial secondary antibodies bound to gold particles are used, secondary antibodies may not have enough specificity to detect your primary antibody in crude sera. Commercial primary antibodies are typically clean enough. The secondary antibodies are usually available commercially at reasonable cost in large amounts.

Fixation

At the fixation stage it is important to preserve the tissue as close to its natural state as possible, while maintaining antigenicity. This is often difficult since the antigenic determinant is easily altered or masked during fixation. It is often best not to use Osmium, however different antigens will not respond uniformly to the fixation processes and experimentation is needed to find the appropriate fixative. Glutaraldehyde, for example, must be used in low concentrations (<1%) as it is a strong denaturing agent for most antigens.

Pre-embedding or Post-embedding Labeling

There are two basic techniques for immuno-EM pre-embedding labeling or post-embedding labelling. For both approaches, the immuno-labelled section must be further stained with heavy metals (such as uranyl acetate) to reveal ultrastructure on the transmission electron microscope.

Pre-embedding labeling involves labeling your cells or tissue with antibodies before it is embedding in a resin. This technique is advantageous for surface labeling of live cells or cell fractions, however the labeling procedure may cause redistribution of the antigen on the surface. Cell interior labeling is also possible but requires detergent extraction to remove or "puncture" the cell membranes so that antibodies can enter the cells. Unfortunately detergents can destroy fine structure in the cells and certain antigens, particularly membrane-bound antigens, can get lost.

Post-embedding labeling is labeling thin sections of cells after they are fixed and embedded in resin. Unlike typical EM resins, this technique usually uses a hydrophilic acrylic resin that is polymerized at low temperature, thus preserving antigenicity (e.g. LR White). Antibody incubations are performed on grid-mounted thin sections (typically nickel grids are used). This technique can give you the best fine structure and morphology because the cells are embedded in a resin. However, the trade-off is that you need an abundant antigen as only antigen on the surface of the section is accessible to the antibody. In addition, some of the chemicals used to embed cells in the resins can denature antigens, further reducing the signal. Sometimes, tissue sections may need to be etched (partially eaten away) with, for example, hydrogen peroxide to expose hidden antigen sites, however care must be taken not to destroy the sections. LR White embedded samples typically do not need etching but epoxy resins do. Typically grids are floated on drops of the various buffer, antibody and blocking solutions which are placed on Parafilm, making sure that the sections do not dry out at any time.

At several steps during a typical protocol the tissue is exposed to normal, or nonimmune serum, or albumen in order to block sites that may react nonspecifically with any antibody (for example non-reacted aldehyde sites). If these steps are not carried out false positives may occur.

Controls

It is essential that immunocytochemical experiments are carried out with a number of controls conducted simultaneously with the localization attempts in order to confirm that the aggregation of label does represent specific localization. In particular use of the tag and unlabeled antibodies separately and omission of the primary or secondary antibodies should be tested to make sure labeling does not occur through some other route.