Protocol

Immunogold Staining of Epoxy Resin Sections for Transmission Electron Microscopy (TEM)

Jeremy N. Skepper and Janet M. Powell

This protocol was adapted from "Ultrastructural Immunochemistry," Chapter 7, in *Immunohistochemistry: Methods Express* (ed. Renshaw), from the *Methods Express* series. Scion Publishing Ltd., Oxfordshire, UK, 2006.

INTRODUCTION

In post-embedding methods of immunogold staining, the cells or tissues are fixed chemically or cryoimmobilized, dehydrated, and embedded in epoxy or acrylic resins. Thin sections (50-70 nm in thickness) are cut using an ultramicrotome with a diamond knife, using a water bath to collect the sections as they slide off the knife. The sections are stretched with solvent vapor or a heat source and collected onto either bare or plastic-coated nickel grids. The sections are then stained immunochemically with primary antibodies raised against antigens exposed on the surface of the sections. The primary antibodies are visualized by staining immunochemically with secondary antibodies raised against the species and isotype of the primary antibodies, conjugated to colloidal gold particles. The immunochemically stained sections are then contrast stained with salts of uranium (uranyl acetate) and lead (lead citrate) to reveal the ultrastructure of the cells, and are finally viewed by transmission electron microscopy (TEM). Chemical fixation and embedding in a highly cross-linked epoxy resin is the method of choice for optimal ultrastructure and stability of the thin section in the electron beam. Immunogold staining of thin epoxy resin sections, described here, is useful if the antigen of interest is very resistant to fixative, or if only archived material that was fixed primarily for ultrastructural studies is available.

RELATED INFORMATION

Ultrastructural Immunochemistry (Skepper and Powell 2008a) describes methods and considerations for the use of immunogold staining, including fixation, controls, resolution, and quantification. The following protocols provide detailed procedures for immunogold staining of various sections for TEM:

Immunogold Staining of London Resin (LR) White Sections for Transmission Electron Microscopy (TEM) (Skepper and Powell 2008b)

Immunogold Staining Following Freeze Substitution and Low Temperature Embedding after Chemical Fixation or after Cryoimmobilization for Transmission Electron Microscopy (TEM) (Skepper and Powell 2008c)

Immunogold Staining of Ultrathin Thawed Cryosections for Transmission Electron Microscopy (TEM) (Skepper and Powell 2008d)

For more comprehensive descriptions of the range of techniques available, see Griffiths et al. (1993) and Skepper (2000).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

<R>Antibodies, primary (optimally diluted in PBSG)

<R>Antibodies, secondary (optimally diluted in PBSG)

Use a secondary antibody raised against the species of the primary antibody and conjugated to 10- or 15-nm colloidal gold particles.

<I>Lead citrate

<I>Methanol (50%, v/v)

<R>PBSG

<I>Periodic acid (aqueous) (1%, w/v)

Aqueous periodic acid is used to remove osmium tetroxide from the surface of thin sections (see Discussion). In some cases, this will enhance the binding of an antibody to its antigen at that surface.

<R>Phosphate-buffered saline (PBS) (pH 7.6)

<I>Potassium hydroxide

Prepare a Petri dish containing a few grains of moistened potassium hydroxide.

<I>Sodium metaperiodate (aqueous) (4%, w/v)

Aqueous sodium metaperiodate is used to remove osmium tetroxide from the surface of thin sections (see Discussion).

Tissue for sectioning (in epoxy resin)

<R>Uranyl acetate (saturated) in 50% methanol

Equipment

Dental wax (or Parafilm)

Dental wax is used as a clean hydrophobic surface on which to perform immunogold staining of thin sections mounted on TEM grids and floated on small drops of reagents.

Diamond trim tool and 45° ultradiamond knife (Diatome AG)

Microscope (transmission electron) (FEI Tecnai 120)

Nickel grids (400 mesh)

Ultramicrotome (EM UCT; Leica Microsystems)

METHOD

All nickel grid incubations/rinses should be performed on dental wax.

1. Cut thin sections (50-70 nm) of tissue in epoxy resin and mount onto nickel grids.
2. Incubate sections on drops of 4% aqueous sodium metaperiodate for 10 min at room temperature.
3. Rinse grids in H₂O for 30-40 sec.
4. Incubate sections on drops of 1% aqueous periodic acid for 10 min.
5. Rinse in H₂O for 30-40 sec.
6. Incubate sections on drops of PBSG for 10 min.
7. Incubate sections overnight on drops of optimally diluted primary antibodies in PBSG.
8. Rinse sections on ten 100-μL drops of 1X PBS for 2 min on each drop.
9. Incubate sections on drops of optimally diluted species-specific secondary antibodies in PBSG (conjugated to 10-or 15-nm gold particles) at room temperature for 2 h.

10. Rinse sections in H_2O for 30-40 sec.
11. Counterstain sections by floating grids, section side down, on drops of uranyl acetate (saturated) in 50% methanol for 0.5-10 min at room temperature. Follow with a rinse in 50% methanol and a rinse in H_2O (Gibbons and Grimstone 1960).
12. Counterstain sections by floating grids, section side down, on drops of lead citrate (Reynolds 1963) for 0.5-10 min at room temperature in a Petri dish containing a few grains of moistened potassium hydroxide (to prevent lead carbonate precipitation).
13. Rinse grids extensively in H_2O and view at 80 kV in a transmission electron microscope.

DISCUSSION

Paraffin wax cannot be used for TEM because it is impossible to cut thin enough sections, since the wax is too soft. Even if it were possible to cut sections that were thin enough, the wax would evaporate in the electron beam and contaminate the column of the microscope. In contrast, immunogold staining of epoxy resin sections allows optimal ultrastructure and stability of the thin section in the electron beam. It would be ideal if we could fix and embed tissue to produce the very best ultrastructure, yet leave the tissue with sufficient antigenicity for it to be immunochemically stained. This would optimally include fixation in a high concentration of glutaraldehyde (2.5% [w/v] or higher), followed by secondary fixation with osmium tetroxide and bulk staining in uranyl acetate. Osmium tetroxide fixes by binding to double bonds in unsaturated fatty acids, retaining them in the subsequent dehydration in organic solvent. It adds positive contrast, because it is a heavy metal that scatters electrons. Similarly, uranyl acetate acts as both a fixative and a stain, because it helps retain phospholipids and adds contrast to the thin sections by scattering electrons. The fixed tissue is dehydrated in an organic solvent infiltrated with an epoxy resin, which is thermally cured at 60°C for up to 48 h. Epoxy resin monomers are joined end to end to form long-chain polymers, which are in turn cross-linked to adjacent polymers during the curing process. This makes them very stable in the transmission electron microscope but hinders access of the antibody to the antigen. Some antigens do survive this treatment, notably small peptide hormones or neurotransmitter substances that are found highly concentrated in secretory vesicles (see Fig. 1). High concentrations of glutaraldehyde are used in protocols for immunochemical staining of amino acid neurotransmitters, such as glutamate and γ -aminobutyric acid (Storm-Mathison and Ottersen 1990). This appears to be necessary to ensure that they are not physically extracted during subsequent dehydration and embedding.

It is generally necessary to remove osmium tetroxide from the superficial regions of the thin section to be immunochemically stained. This is readily achieved by treatment with one or a combina-

FIGURE 1. Thin section through a rat pancreatic β -cell. The section was fixed in 4% glutaraldehyde/1% osmium tetroxide, bulk stained in uranyl acetate, and embedded in Spurr's resin. The section was treated with sodium metaperiodate before immunolabeling for insulin. The crystalline cores of the secretory granules are heavily labeled with gold particles. Bar, 250 nm. (Reprinted with permission from Scion Publishing Ltd. © 2006.)

tion of the following oxidizing agents: 10% (v/v) hydrogen peroxide (Causton 1984), 4% (w/v) sodium metaperiodate (Bendayan and Zollinger 1983), or 1% (w/v) periodic acid (Storm-Mathison and Ottersen 1990). This pretreatment of resin sections of tissues fixed to maximize ultrastructural preservation has been used to great effect for the study of secretory proteins, peptides, and neurotransmitters (Probert et al. 1981; Bendayan et al. 1986; Varndell et al. 1986; Skepper et al. 1988; Newman et al. 1991). However, these antigens are generally present in very high local concentrations within secretory granules. Oxidizing agents attack the hydrophobic alkane side-chains of epoxy resins, which make the sections more hydrophilic (Causton 1984). This allows more intimate contact between the immunochemical reagents and the antigens exposed at the surface of the sections.

REFERENCES

- Bendayan, M. and Zollinger, M. 1983. Ultrastructural localization of antigenic sites on osmium-fixed tissues applying the protein A-gold technique. *J. Histochem. Cytochem.* **31**: 101–109.
- Bendayan, M., Nanci, A., Herbener, G.H., Gregoire, S., and Duhr, M.A. 1986. A review of the study of protein secretion applying the protein A-gold immunocytochemical approach. *Am. J. Anat.* **175**: 379–400.
- Causton, B. 1984. The choice of resins for electron immunocytochemistry. In *Immunolabelling for electron microscopy* (eds. J.M. Polak and I.M. Varndell), pp. 29–36. Elsevier, Amsterdam.
- Gibbons, I.R. and Grimstone, A.V. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* **7**: 697–716.
- Griffiths, G., Burke, B., and Lucocq, J. 1993. *Fine structure immunocytochemistry*. Springer-Verlag, Heidelberg, Germany.
- Newman, T.M., Severs, N.J., and Skepper, J.N. 1991. The pathway of atrial natriuretic peptide release—from cell to plasma. *Cardioscience* **2**: 263–272.
- Probert, L., De Mey, J., and Polak, J.M. 1981. Distinct subpopulations of enteric p-type neurones contain substance P and vasoactive intestinal polypeptide. *Nature* **294**: 470–471.
- Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**: 208–212.
- Skepper, J.N. 2000. Immunocytochemical strategies for electron microscopy: Choice or compromise. *J. Microsc.* **199**: 1–36.
- Skepper, J.N. and Powell, J.M. 2008a. Ultrastructural immunocytochemistry. *CSH Protocols* (this issue) doi: 10.1101/pdb.top47.
- Skepper, J.N. and Powell, J.M. 2008b. Immunogold staining of London Resin (LR) White sections for transmission electron microscopy (TEM). *CSH Protocols* (this issue) doi: 10.1101/pdb.prot5016.
- Skepper, J.N. and Powell, J.M. 2008c. Immunogold staining following freeze substitution and low temperature embedding after chemical fixation or after cryoimmobilization for transmission electron microscopy (TEM). *CSH Protocols* (this issue) doi: 10.1101/pdb.prot5017.
- Skepper, J.N. and Powell, J.M. 2008d. Immunogold staining of ultrathin thawed cryosections sections for transmission electron microscopy (TEM). *CSH Protocols* (this issue) doi: 10.1101/pdb.prot5018.
- Skepper, J.N., Woodward, J.M., and Navaratnam, V. 1988. Immunocytochemical localization of natriuretic peptide sequences in the human right auricle. *J. Mol. Cell. Cardiol.* **20**: 343–353.
- Storm-Mathisen, J. and Ottersen, O.P. 1990. Immunocytochemistry of glutamate at the synaptic level. *J. Histochem. Cytochem.* **38**: 1733–1743.
- Varndell, I.M., Sikri, K.L., Hennessy, R.J., Kalina, M., Goodman, R.H., Benoit, R., Diani, A.R., and Polak, J.M. 1986. Somatostatin-containing D cells exhibit immunoreactivity for rat somatostatin cryptic peptide in six mammalian species. An source-microscopical study. *Cell Tissue Res.* **246**: 197–204.

Cold Spring Harbor Protocols

Immunogold Staining of Epoxy Resin Sections for Transmission Electron Microscopy (TEM)

Jeremy N. Skepper and Janet M. Powell

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot5015

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

- [Antibodies, general](#) (289 articles)
- [Cell Biology, general](#) (1382 articles)
- [Cell Imaging](#) (525 articles)
- [Electron Microscopy](#) (43 articles)
- [Imaging/Microscopy, general](#) (579 articles)
- [Immunohistochemistry](#) (92 articles)
- [Immunoinmaging](#) (41 articles)
- [Immunology, general](#) (128 articles)
- [Immunostaining](#) (139 articles)
- [Immunostaining Cells](#) (51 articles)
- [Labeling for Imaging](#) (339 articles)
- [Phenotypic Analysis](#) (42 articles)
- [Visualization](#) (524 articles)
- [Visualization of Gene Expression](#) (127 articles)
- [Visualization of Organelles](#) (88 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>