Detection of protein expression by immunocytochemistry

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Level of Resolution?

- General area in a tissue
- Which cell type?
- Organelle (membrane, cytosol, organelle, nucleus?)
- Colocalization
- Sidedness on membrane
Detection of GFP in a transgenic mouse kidney using thick vibratome section (left) and 5 µm cryostat section (right)

GFP (PC) - green
V-ATPase (IC) - red
Double immunofluorescence staining - 5 µm cryostat section

PC - AQP Basolateral

IC - PP Apical
Golgi localization of Arf1 GTPase in LLC-PK1 cultured cells
Immunogold showing sidedness of epitopes

External domain antibody

Lumen

Cytosol
Tissue/cell preparation - effects of fixation

1. **No fixation - snap freezing**

2. **Light fixation**
   - Acetone/methanol
   - Precipitating fixatives (Carnoy’s, Bouin’s solutions)
   - 2-4% paraformaldehyde

3. **Intermediate fixation**
   - Paraformaldehyde/lysine/periodate
   - 4% paraformaldehyde/0.1% glutaraldehyde

4. **Stronger fixation**
   - 1-4% glutaraldehyde (for EM structure)
Embedding/sectioning procedures

1. **Light Microscopy**
   - Frozen sections - 5 µm cryostat or 1 µm “semithin”
   - Paraffin-embedded sections
   - Resin-embedded sections (Epon, hydrophilic resins)
   - Whole mounts, cell cultures on coverslips/filters
   - Thick vibratome sections

2. **Electron Microscopy**
   - Frozen sections - ultracryomicrotomy
   - Resin embedded sections - K4K, HM20, LR resins
   - Epon-embedding after pre-embedding labeling with peroxidase or gold
Double immunofluorescence staining - 5 µm cryostat section

PC - AQP Basolateral

IC - PP Apical
SEMITHIN (1 MICRON) CRYOSTAT SECTION - AQP2/AE1
Whole-mount of vas deferens stained for proton pumps
Detection of cytosolic CaBP by immunogold on K4M sections
AQP2 on apical surface of principal cells using antibody against the external domain of AQP2

Pre-embedding gold labeling of tissue

Epon embedding

Note absence of intracellular label (gold)
Which technique do I use?

1. **Light microscopy**
   - Fluorescence
   - Peroxidase or other enzymatic method
   - Amplification techniques (e.g., NEN-tyramide kit)

2. **Confocal microscopy**
   - Conventional laser confocal (stacks, rotations)
   - Dual-photon confocal (increased tissue depth)
   - Spinning disk confocal (live cell imaging)

3. **Electron microscopy**
   - Immunoperoxidase
   - Immunogold
Triple immunofluorescence staining - 5 µm cryostat section

- FITC-dextran (green)
- AE1 (blue)
- Proton pump (red)
- Proton-pump And FITC-dextran (yellow)
- V-ATPase (red)
Tyramide amplification to detect antigens in different cells using antibodies raised in the same species.

1st primary used very dilute, then amplified using FITC kit.

2nd primary used at normal dilution and detected with IgG-CY3.
Tyramide amplification

HRP activity $\rightarrow$ Ty-F + any protein

$\text{H}_2\text{O}_2$

Conventional Indirect IF

2 antibody

1 antibody

antigen

Activated Ty-F is stable for only a short time - restricted diffusion
Immunogold labeling of proton pumps on microvilli - internal domain

(Lowicryl-K4M Embedding)
Calbindin 28 in “Principal cells” of rat kidney

Immunogold on K4M
### Hints/Tips/Troubleshooting

**Increasing weak signals**

- Concentration of antibody
- Brighter fluorophore
- Longer incubation
- Amplification
- Digital enhancement
Digital Enhancement (Adobe Photoshop “levels” command)
Effect of initial image resolution on enlargement quality.
Antigen Retrieval Techniques

- Sodium dodecyl sulfate
- Microwaves plus acidic buffer
- Protease digestion
- Detergents (TX-100, saponin)
Antigen retrieval using pre-Treatment of tissue sections
With SDS

Syntaxin 3 staining in kidney

With SDS

No SDS

Brown et al. (1996)
Histochem Cell Biol, 105: p261
Hints/Tips/Troubleshooting

**Reducing Background**

- Quench aldehydes (NH₄Cl)
- Protein/IgG blocking solutions
- High salt PBS (2.7% NaCl)
- Centrifuge antibodies
- Sharp microtome blades
- Other secondary reagents
Use of Evans Blue counterstain in immunofluorescence
Use of Photoshop levels for background removal
Digital Deconvolution - removes haze and sharpen image
**Hints/Tips/Troubleshooting**

**Controls**

- Omit primary antibody
- Pre-immune serum
- Antigen/peptide inhibition
- Affinity purification of IgG
- Western blotting
- Other antibodies vs same protein
- Knockout mice
Thank You

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