—Photogravure—

Multiple Immunofluorescence Labeling in Tissue Sections

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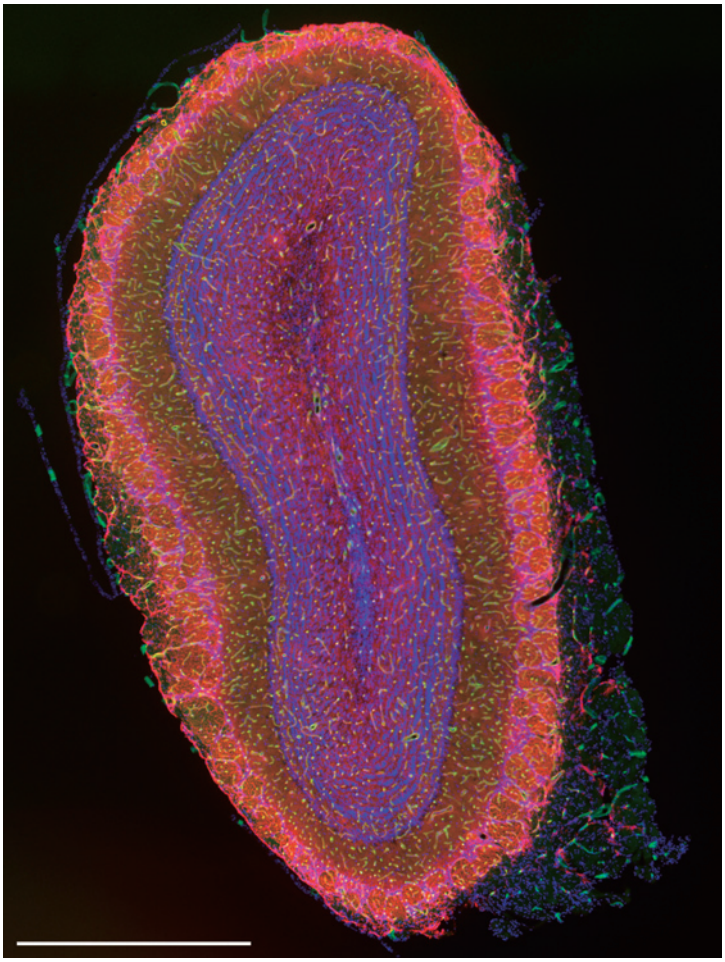

Fig. 1

Immunofluorescence microscopy is a commonly used histochemical or cytochemical technique to detect interesting molecules. Combinations of primary antibodies raised in different animal species and appropriate secondary antibodies enable multiple immunofluorescence labeling. Here we show 2 examples. **Figure 1** is an example of triple labeling with 2 primary antibodies raised in a rabbit and a guinea pig as well as DNA-binding fluorochrome. **Figure 2** shows an example of double labeling with a primary antibody and fluorochrome-conjugated actin-binding protein.

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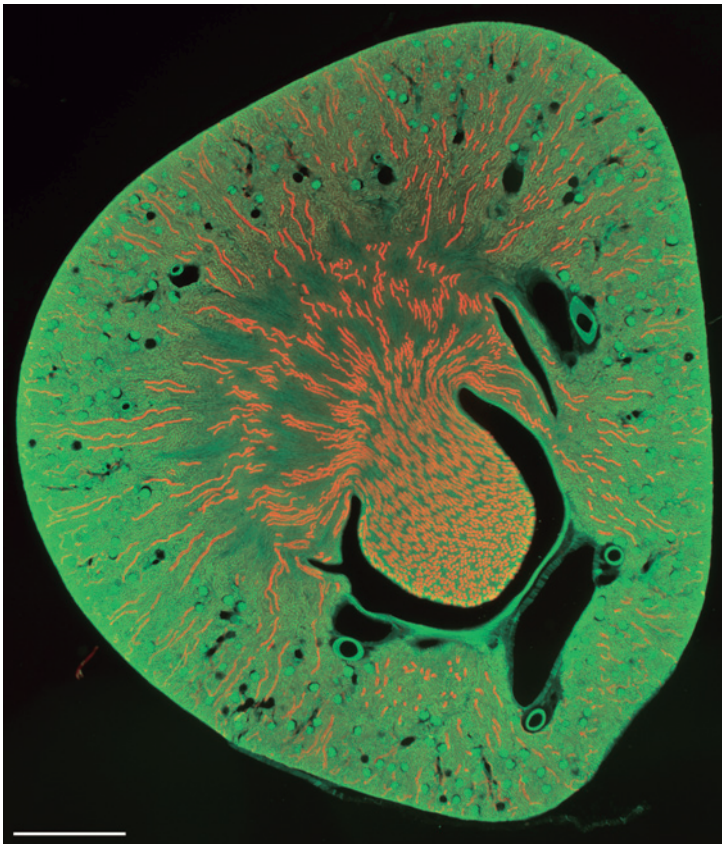

Fig. 2

Fig. 1 Triple labeling for water channel AQP4, glucose transporter GLUT1, and cell nuclei in the rat olfactory bulb

A rat brain was perfusion fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH7.4. A free floating cryostat section (20 μm thick) was incubated with a mixture of rabbit anti-AQP4 antibody and guinea pig anti-GLUT1 antibody. Secondary antibodies used were Rhodamine Red-X-conjugated donkey anti-rabbit antibody and fluorescein isothiocyanate (FITC)-conjugated donkey anti-guinea pig antibody. For nuclear counterstaining, 4',6-diamidino-2-phenylindole (DAPI) was added to the mixture of secondary antibodies. AQP4 (red) is distributed in astrocytes and plays an important role in water homeostasis in the brain. GLUT1 (green) is localized to the endothelial cells of the blood-brain barrier and plays a pivotal role in the transfer of glucose across the barrier. Cell nuclei labeled with DAPI are shown in blue. Bar, 1 mm.

Fig. 2 Double labeling for water channel AQP2 and filamentous actin in the rat kidney

A rat kidney was perfusion fixed and a cryostat section (50 μm thick) was cut. A free-floating tissue section was subjected to indirect immunofluorescence staining with rabbit anti-AQP2 antibody and Rhodamine Red-X-conjugated donkey anti-rabbit antibody. Filamentous actin was stained with Alexa Fluor 488-conjugated phalloidin, an actin-binding protein. Glomeruli and brush borders of proximal tubules are clearly shown by phalloidin staining (green). Vasopressin-regulated water channel AQP2 (red) is localized to the connecting tubules as well as collecting ducts, and plays a critical role in water reabsorption. Bar, 1 mm.

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