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Double immunostaining for PIP2 and phosphorylated ERK

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Protocol Information

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Summary: Immunofluorescence microscopy is used to determine molecular localization and to measure fluorescence intensity of activity in cultured cells. Reagents for fixation and/or permeabilization must be suitable for the molecule of interest. We developed a procedure to immunostain phosphatidylinositol biphosphate (PIP2) and phosphorylated Extracellular signal-Regulated Kinase (ERK) simultaneously. PIP2 is a phospholipid component of cell membranes. ERK is a mitogen-activated protein kinase (MAPK) that localized in the cytoplasm and nucleus after phosphorylation triggered by ligand activation. Different compounds are used to permeabilize cells in order to stain for these two molecules. Digitonin is used for PIP2 and Triton X-100 is used for phosphorylated ERK. However, since Triton X-100 can solubilize cell membrane lipids, it is not suitable for PIP2 labeling. In order to confer resistance against Triton X-100, we devised a method to fix anti-PIP2 antibody on the cell membrane antigen before permeabilization with Triton X-100. We modified a conventional immunostaining method to include secondary fixation and permeabilization after anti-PIP2 antibody labeling. The secondary fixation probably immobilizes antibodies bound to PIP2 on the cell membrane, perhaps by cross-linking them to surrounding protein molecules. Whatever the mechanism, this fixation enables PIP2-antibody complexes to remain on the membrane, despite treatment with Triton X-100, which is used to visualize phosphorylated ERK. Since PIP2 and ERK are major cell signaling molecules in various cellular phenomena, simultaneous detection and visualization of their phosphorylation states by this method should be useful for many studies.

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