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Immunolabeling of endogenous proteins for fluorescence microscopy in cultured mammalian cells

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We use this protocol and it's working

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Abstract

This protocol details the detection of endogenous proteins by immunofluorescence. This is our standard protocol for imaging endogenous proteins associated with mitochondria and other membrane-bound organelles.

Guidelines

Introduction

This protocol provides detailed instructions for detection of endogenous proteins in fixed cells by immunofluorescence (Immunofluorescence Assay/IFA). The protocol results in a fluorescent sample, to be visualized using a high-resolution fluorescence confocal microscope. Target proteins appear as distinct signals corresponding to the fluorophore-conjugated secondary antibodies, allowing the spatial analysis of subcellular structures, protein interactions, and nucleic acids.

Materials

- Proliferating cancer cell line or fibroblasts at 40% confluency
- 1X Phosphate-buffered saline (PBS), pH 7.4
- Fixative: 4% Paraformaldehyde in PBS, pH 7.4
- Quenching Solution: 1M Glycine in PBS, pH 7.4
- Permeabilization Solution: 0.1% Triton-X 100 in PBS, pH 7.4
- Blocking Solution: 0.1% Tween in 1X TBS + 1% BSA (Bovine Serum Albumin)
- Glass bottom poly-D-lysine-coated dishes (P35GC-1.5-14-C, MatTek)
- Primary antibody (specific to the target protein)
- Secondary antibody conjugated to fluorophore of choice

Method

4d 1h 1m

1 Prepare the sample by plating cells in 2 mL of media at a 20% density in a glass bottom poly-D-lysine-coated dish (P35GC-1.5-14-C, MatTek).

2 Grow cells for 24:00:00 until they reach a confluency of approximately 40%. 1d

3 Aspirate media and fix the cells with 2 mL of pre-warmed 4% PFA in PBS.

4 Incubate for 00:20:00 at Room temperature on a shaker gently rotating. 20m

5 Quench the reaction by adding 200 µL of 1M glycine in PBS to the fixed sample and incubate for 00:01:00 at Room temperature. 1m

6 Aspirate the quenched fixative and wash the sample once with PBS.

7 Add 2 mL of 0.1% Triton X-100 in PBS and incubate for 00:10:00 at Room temperature on a shaker to permeabilize the sample. 10m

8 Aspirate permeabilized solution and wash sample once with PBS.

9 Add 2 mL of blocking buffer (1% BSA + 0.1% Tween in 1X TBS) with a 00:10:00 incubation at Room temperature on a shaker. 10m

10 Aspirate blocking buffer and add a freshly prepared 2 mL primary antibody dilution (typically a 1:1000 dilution) made in blocking buffer.

11 Incubate the sample Overnight at 4 °C in the dark.

12 Aspirate the primary antibody solution and wash the sample once with 2 mL of blocking buffer.

13 Aspirate wash and add 2 mL of freshly prepared secondary antibody dilution (typically at a 1:2000 dilution) with a 00:10:00 incubation at Room temperature on a shaker in the dark. 10m

14 Aspirate the secondary antibody solution and wash the sample with 2 mL of blocking buffer with a 00:10:00 incubation at Room temperature on a shaker. 10m

15 Remove wash and cover sample with 2 mL PBS.

16 Proceed with fluorescence microscopy.

17 Samples may be stored at 4 °C protected from light for up to 72:00:00; bring dishes to Room temperature before imaging. 3d

Protocol references

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