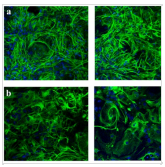

Immunocytochemistry

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Working

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ABSTRACT

Immunocytochemistry for morphology analysis. Staining of cell culture in order to obtain fluorescence images of the culture.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Cell fix

1 1. Wash the culture twice in a phosphate buffered saline (PBS);
2. Fix with 4% paraformaldehyde (Merck) for 10 min 00:10:00 , then put it in PBS until staining.

Staining

2 Wash fixed cultures three times with PBS (10 min 00:10:00 each).

Permeabilization

3 Permeabilize with 0.5% triton X-100 (Sigma-Aldrich) in PBS for 10 min 00:10:00 .

Block

4 Block with 2% BSA, 10% normal donkey serum, and 0.25% triton X-100 solution in PBS for 1 h 01:00:00 at room temperature.

Primary antibody incubation

5 Incubate overnight with rabbit anti-GFAP (1:400, Sigma-Aldrich, cat# G9269, RRID: AB_477035), mouse anti-NeuN (1:200, Millipore, cat# MAB377, RRID: AB_2298772) at 4 °C 4 °C .

Secondary antibody incubation

6 Wash three times with PBS and incubated for 1 h 01:00:00 at room temperature with the appropriate secondary antibodies: Alexa fluor 488 goat anti-rabbit IgG (1:400, Jackson Laboratories) for the detection of GFAP and Cy-3 donkey anti-mouse IgG (1:700, Jackson Laboratories) for NeuN.

DAPI stain

7 Mount with aqueous medium containing DAPI (VECTASHIELD Mounting Medium with DAPI, Vector Laboratories, H-1200).