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Immunofluorescence and confocal microscopy

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Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

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We use this protocol and it's working

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Abstract

This protocol details the process of immunofluorescence and confocal microscopy.

Materials

28906, Thermo Fisher Scientific

Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific Catalog #**28906

0.1% (v/v) Triton X-100 (9002-93-1, Sigma-Aldrich)

5% (v/v) BSA (9048-46-8, Sigma-Aldrich) Bovine Serum Albumin [BSA] **Fisher Scientific Catalog #**9048-46-8

DAPI Fluoromount-G mounting medium (0100-20, Southern Biotech)

Steps

2h 15m

1 Seed the cells on glass coverslips (12 mm #1.5) at a concentration of 100.000 cells/well, and after treatment with Rapalog for the indicated time, fix in 4% paraformaldehyde (28906, Thermo Fisher Scientific) for 00:10:00 at Room temperature . — 10m

2 After washing with PBS, permeabilize the cells with 0.1% (v/v) Triton X-100 (9002-93-1, Sigma-Aldrich) in PBS for 00:05:00 . — 5m

3 Perform the blocking with blocking buffer (5% (v/v) BSA (9048-46-8, Sigma-Aldrich) and 0.05% (v/v) Triton X-100 diluted in PBS) for 01:00:00 at Room temperature . — 1h

4 Dilute the primary and secondary antibodies in blocking buffer and incubate for 01:00:00 at Room temperature with three PBS washing steps in between. — 1h

5 Mount the cells on microscopy slides in DAPI Fluoromount-G mounting medium (0100-20, Southern Biotech), which stains the nuclei, and store at 4 °C until use.

6 Perform the confocal microscopy with a Zeiss LSM700 laser scanning confocal microscopy with Plan-Apochromat 40×/1.30 Oil DIC, WD 0.21 mm objective.