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Immunofluorescence - RAW 264.7

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We use this protocol and it's working

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Abstract

This protocol details steps taken to perform immunofluorescence in RAW 264.7 cells.

Materials

DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomycin (Thermo Fisher Scientific, 15140122)
CellStripper (Corning, 356230)
PBS [1.1 mM KH2PO4 (J.T. Baker, 3246-01), 155.2 mM NaCl (Sigma-Aldrich, 3624-05), and 3 mM Na2HPO4 (J.T. Baker, 3828-05)]
Paraformaldehyde (Electron Microscopy Sciences, 19202)
Sodium phosphate buffer (pH 7.3 / Buffer: 153.56 mM Sodium phosphate, dibasic, anhydrous, J.T. Baker 3828, 53.63 mM Sodium dihydrogen phosphate monohydrate, J.T. Baker 3818)
Sucrose (Sigma-Aldrich, S0389)
BSA (Sigma-Aldrich, A9647)
Saponin (Sigma-Aldrich, S4521)
Triton (Sigma-Aldrich, X100)
Coverslips (12 mm, Carolina Biological Supplies, 633029)
Microscope Slides (Thermo Fisher Scientific, 12-550-143)
Prolong Gold Mounting Media (Thermo Fisher Scientific, P36935)

Day 0

1 Plate 2 × 10^5 RAW 264.7 cells on poly-D-lysine coated coverslips.

Day 1

35m

2 Add the indicated compound at the provided concentration and time point in fresh DMEM media containing FBS and P/S.

2.1 Proceed to next step if not treating cells.

3 Fix cells in 4% paraformaldehyde supplemented with 4% sucrose for 00:30:00 at Room temperature . 30m

4 Wash coverslips 3X for 00:05:00 with PBS at Room temperature . 5m

5 Add primary antibodies Overnight at 4 °C in 3% BSA in PBS.

Day 2

1h 10m

6 Wash coverslips 3X for 00:05:00 with PBS at room temperature. 5m

7 Add secondary antibody for 01:00:00 at Room temperature in PBS in the dark. 1h

8 Wash coverslips 3X for 00:05:00 with PBS at Room temperature . 5m

9 Mount coverslips onto microscope slides with Prolong Gold mounting media and store at 4 °C .

Day 3+

10 Image coverslips.