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Immunocytochemistry Staining Protocol

Forked from Immunocytochemistry Staining Protocol

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Simeng Li[1]

[1]University College Dublin

Simeng Li

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External link:
http://www.biolegend.com/media_assets/support_protocol/Immunofluorescence_Microscopy_Protocol_050514.pdf

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Protocol status: In development
We are still developing and optimizing this protocol

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Troubleshooting

Before start

Reagent List:

	▪ Chamber slides, cover slips, or 12-well plates ▪ Phosphate-buffered saline (PBS)	▪ Fixation solution: 1% Paraformaldehyde, in PBS ▪ Permeabilization solution: 0.5% Triton X-100 in PBS ▪ Blocking buffer: 5% FBS in PBS

Sample Preparation

1 Grow cultured cells on cover slips or in wells overnight at 37°C. At the time of fixation, cells should be ~70-80% confluent in single layer.

2 Rinse cells briefly in PBS.

3 Fix cells by incubation with freshly made 4% Paraformaldehyde in PBS for 15 minutes at room temperature.
00:15:00

4 Rinse three times quickly in PBS.

Sample Blocking

5 Block samples in 1 mL of blocking buffer at room temperature for 1 hour.
00:30:00

Sample Staining

6 Dilute the primary antibody to the recommended concentration/dilution in blocking buffer.

7 For 8-well chamber slides, add 200 µL per well. For 12-well plates, add 500 µL per well. Incubate two to three hours at room temperature or overnight at 4°C. If using conjugated antibodies, perform this step in the dark.

8 For surface staining, rinse 3 times quickly in PBS.
00:10:00

9 Prepare fluorochrome-conjugated secondary antibody in blocking buffer according to the manufacturer's specification data sheet, and add 200 µl per well to the 8-well chamber slides. For 12-well plates, add 500 µL per well.

10 Incubate the samples for one hour, at room temperature, in the dark.
01:00:00

11 For surface staining, rinse three times quickly in PBS.
00:10:00

12 Counterstain with DAPI for 15 minutes at 37 ℃.

13 Wash cells 3 times and the third time keep PBS in the wells.

14 Image CD31 at 488nm and DAPI at 388nm