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# Immunocytochemistry Staining Protocol

 Forked from [Immunocytochemistry Staining Protocol](#)

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**External link:**

[http://www.biolegend.com/media\\_assets/support\\_protocol/Immunofluorescence\\_Microscopy\\_Protocol\\_050514.pdf](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**

**Created:** September 12, 2018

**Last Modified:** September 12, 2018


**Protocol Integer ID:** 15638

## Before start


### Reagent List:

<ul style="list-style-type: none"><li>▪ Chamber slides, cover slips, or 12-well plates</li><li>▪ Phosphate-buffered saline (PBS)</li></ul>	<ul style="list-style-type: none"><li>▪ Fixation solution: 1% Paraformaldehyde, in PBS</li><li>▪ Permeabilization solution: 0.5% Triton X-100 in PBS</li><li>▪ Blocking buffer: 5% FBS in PBS</li></ul>
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## 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 °C.
- 13 Wash cells 3 times and the third time keep PBS in the wells.
- 14 Image CD31 at 488nm and DAPI at 388nm