SEP 12, 2018

Immunocytochemistry Staining Protocol

Forked from [Immunocytochemistry Staining Protocol](#)

Simeng Li¹

¹University College Dublin

Simeng Li

BEFORE START INSTRUCTIONS

Reagent List:

■	Fixation solution: 1% Paraformaldehyde, in PBS
■	Chamber slides, coverslips, or 12-well plates
■	Phosphatase

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.thwej7e

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

Protocol Citation: Simeng Li 2018.
Immunocytochemistry Staining Protocol.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.thwej7e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Sep 12, 2018

Last Modified: Sep 12, 2018

h a t e- b u f f e r e d s a l i n e (P B S)	■	T r i t o n X- 1 0 0 i n P B S B l o c k i n g b u f f e r: 5 % F B S i n P B S
--	---	---

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 μ L per well. For 12-well plates, add 500 μ L 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 μ L per well to the 8-well chamber slides. For 12-well plates, add 500 μ L 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