Oct 11, 2024 Version 2

Flow Cytometry Protocol for LAMP1 Staining V.2

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvr8b6blmk/v4

Ali Ghoochani[1,2,3,4], Monther Abu-Remaileh[1,2,3,4]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA;
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA;
[3]The Institute for Chemistry, Engineering and Medicine for Human Health (Sarafan ChEM-H), Stanford University, Stanford, CA 94305, USA;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

asap

Ali Ghoochani
Stanford University

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvr8b6blmk/v4

Protocol Citation: Ali Ghoochani, Monther Abu-Remaileh 2024. Flow Cytometry Protocol for LAMP1 Staining. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.6qpvr8b6blmk/v4**Version created by **Ali Ghoochani**

Protocol status: Working
We use this protocol and it's working

Created: October 11, 2024

Last Modified: October 11, 2024

Protocol Integer ID: 109683

Keywords: flow cytometry protocol for lamp1, staining of lysosome, lysosome, flow cytometry, detailed procedure for flow cytometry, flow cytometry protocol, associated membrane protein, membrane protein, lamp1, cytometry, membrane, human cell, cell

Abstract

This protocol outlines a detailed procedure for flow cytometry-based staining of lysosome-associated membrane protein 1 (LAMP1) in human cells.

Materials

- Trypsin
- 3% Formaldehyde in PBS
- 90% Ice-cold methanol
- 3% BSA in PBS
- LAMP1 antibody (Rabbit anti-human LAMP1, Cell Signaling Technology, Cat. No. 9091S)
- Alexa Fluor-647 secondary antibody
- Wash buffer: PBS + 1% BSA + 0.05% Tween-20
- Flow cytometer
- Centrifuge

Cell Lifting and Centrifugation

1 Lift adherent cells by adding **trypsin** to the culture plate.

1.1 Once cells are detached, centrifuge the cells at ~ 1000 x g, 00:03:00 to pellet them. 3m

Cell Fixation

2 Resuspend the cell pellet in 1 mL of 3% formaldehyde.

2.1 Incubate the cells at 3 37 °C for 00:10:00 to fix cells. 10m

2.2 Centrifuge the cells at 1000 x g for 00:03:00 and discard the supernatant. 3m

Permeabilization

3 Resuspend the cell pellet in 1 mL 90% ice-cold methanol while vortexing at low speed to prevent clump formation.

3.1 After the incubation, centrifuge the cells at 1000 x g, 4°C, 00:03:00 and discard the supernatant. 3m

3.2 Wash cell pellet with 1 mL 1XPBS. 1000 x g, 4°C, 00:03:00 and and discard the supernatant. 3m

Blocking

4 Resuspend the cells in 1 mL of 3% BSA (in PBS).

4.1 Incubate at 37 °C for 00:30:00 to block non-specific binding sites. 30m

4.2 Centrifuge the cells at 1000 x g, 00:03:00 and discard the supernatant. 3m

Primary Antibody Staining

5 Resuspend the cells in 3% BSA in PBS containing a 300 µL 1:1000 dilution of Rabbit anti-human LAMP1 antibody (Cat. No. 9091).

5.1 Incubate at 37 °C, for 01:00:00 to 02:00:00 with intermittent low-speed vortexing to keep the cells in suspension. 3h

5.2 Centrifuge the cells at 1000 x g, 00:03:00 and discard the supernatant. 3m

5.3 Resuspend the cells in wash buffer (PBS + 1% BSA + 0.05% Tween-20) and centrifuge at 1000 x g, 00:03:00 and discard the supernatant. 3m

5.4 Repeat the wash step once more to remove excess primary antibody. 1000 x g, 00:03:00 and discard the supernatant. 3m

Secondary Antibody Staining

6 Resuspend the cells in 3% BSA in PBS containing a 1:1000 dilution of Alexa Fluor-647 (or any fluorescently labeled secondary antibody compatible with the flow cytometer).

6.1 Incubate at 37 °C for 00:30:00 to 01:00:00 1h 30m

6.2 Wash the cells twice with wash buffer to remove unbound secondary antibody. Centrifuge the cells at 1000 x g, 00:03:00 and discard the supernatant. 3m

Flow Cytometry

7 Resuspend the cells in an appropriate volume of wash buffer (Usually 500 µL for flow cytometry analysis.

8 Measure the intensity of LAMP1 expression using a flow cytometer, detecting the Alexa Fluor-647 signal (or equivalent secondary antibody fluorophore).