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# Measuring lysosomal exocytosis by Flow cytometry

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

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## Abstract

Protocol for measuring lysosomal exocytosis by flow cytometry.

## Materials

**Staining buffer:** 5% FCS in PBS (filtered)
**Blocking buffer:** 5% FCS in PBS containing FcR block at 1:100 dilution (Anti-Mouse CD16/CD32; BD Biosciences Cat# 553141, RRID:AB_394656)
**Antibody staining solution:** Dilute 0.5 ul anti-mouse-LAMP1-AF647 (Rat-anti-mouse-LAMP-1-AF647 (BioLegend Cat# 121610, RRID:AB_571990) ) in 25 ul staining buffer per sample.
**Viability stain:** eg Fixable Viability Dye eFluor-450, # 65-0863-18, eBioscience

## Sample preparation

1 Seed cells as required and stimulate to induce exocytosis. Prepare ~ 5×10^5 cells per sample.

2 Detach cells. **Keep all samples on ice from now on.**

## Staining

3 Pellet cells.

4 Resuspend in 75 ul blocking buffer (5% FCS in PBS - optional: containing FcR blocker) and incubate for 10 min on ice.

5 Add 25 ul anti-LAMP1-AF647 antibody staining solution containing 0.5 ul antibody and incubate for 30 min on ice. Protect from light.

6 Add 300 ul PBS and pellet cells.

7 Resuspend cells in 100 ul dead/live staining solution and incubate for 10 min on ice. Protect from light.

8 Add 300 ul PBS and pellet cells.

9 Resuspend cells in 300 ul 2 % PFA/PBS.

## Sample aquisition and analysis

10 Acquire 10'000 events per sample.

11 After removing doublets, analyse the mean and medium LAMP1 fluorescence intensity in the live cell gate.

## Controls

10m

12 **Dead/live single-colour control**

10m

To prepare a live/dead single stain control, heat half of the sample at 65 °C for 00:10:00 min. Put back on ice immediately, mix with non-heat treated cells and stain as above.

**LAMP1 single-colour control**

Remove part of the stimulated samples and omit dead/live stain.