



Dec 15, 2023

Flow cytometry for ex vivo stimulated pMacs

 [Molecular Neurodegeneration](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzx9x4gx1/v1>

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DOI: <https://dx.doi.org/10.17504/protocols.io.rm7vzx9x4gx1/v1>

External link: <https://doi.org/10.1186/s13024-025-00829-w>

Protocol Citation: Rebecca Wallings 2023. Flow cytometry for ex vivo stimulated pMacs. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.rm7vzx9x4gx1/v1>

Manuscript citation:

Wallings RL, Gillett DA, Staley HA, Mahn S, Mark J, Neighbarger N, Kordasiewicz H, Hirst WD, Tansey MG (2025) ASO-mediated knock-down of GPNMB in mutant-GRN and in Grn-deficient peripheral myeloid cells disrupts lysosomal function and immune responses. *Molecular Neurodegeneration* 20(). doi: [10.1186/s13024-025-00829-w](https://doi.org/10.1186/s13024-025-00829-w)

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Protocol status: Working

We use this protocol and it's working

Created: December 14, 2023

Last Modified: December 15, 2023

Protocol Integer ID: 92326

Keywords: pmacs flow cytometry for ex vivo, pmacs flow cytometry, cytometry, pmac

Funders Acknowledgements:

ASAP

Grant ID: ASAP-020527

Abstract

Flow cytometry for ex vivo stimulated pMacs

- 1 Incubate cells for 30 minutes at 37 degrees in incubator in media containing 3mM EDTA at pH 6.2 to gently lift cells from plate. To encourage dissociation from plate, gently tap plate, or alternatively use a mini scraper
- 2 Aliquot cells in to wells of v-bottom 96 well plate

Spin cells at 300 x g at 4 degrees for 5 minutes

Resuspend cells in 50uL of antibody cocktail containing live/dead dye and Fc block

Incubate at 4 degrees in the dark for 20 minutes

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL 1 x PBS

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL 1 x PBS

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 50uL ICC fixation buffer (Invitrogen)

Incubate cells at room temperature for 10 minutes in the dark

Spin cells at 300 x g at 4 degrees for 5 minutes

Remove supernatant and resuspend pellet in 200uL FACS buffer and proceed to assess cells on cytometer