



Sep 29, 2025

## Microglia validation by FACS-analysis

DOI

[dx.doi.org/10.17504/protocols.io.81wgbxokqlpk/v1](https://dx.doi.org/10.17504/protocols.io.81wgbxokqlpk/v1)

Riana Lo Bu<sup>1,2</sup>, Frank Soldner<sup>1,2</sup>

<sup>1</sup>Albert Einstein College of Medicine, 1301 Morris Park Ave., Bronx, NY 10461, USA.;

<sup>2</sup>Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815.



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DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbxokqlpk/v1>

**Protocol Citation:** Riana Lo Bu, Frank Soldner 2025. Microglia validation by FACS-analysis. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.81wgbxokqlpk/v1>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** January 09, 2024

**Last Modified:** September 29, 2025

**Protocol Integer ID:** 93109

**Keywords:** ASAPCRN, microglia validation by fac, microglia validation, microglia validation by fluorescence, microglia differentiation protocol, procedure for microglial precursor, microglia differentiation protocol for cell generation, microglial precursor, microglia differentiation, activated cell sorting, cell sorting, fac, list of reagent

### **Funders Acknowledgements:**

**Aligning Science Across Parkinson's (ASAP)**

Grant ID: ASAP-000486

**Aligning Science Across Parkinson's (ASAP)**

Grant ID: ASAP-024409

## Abstract

This protocol describes the procedure for microglial precursor and mature microglia validation by Fluorescence-Activated Cell Sorting (FACS) analysis. Please refer to (<https://doi.org/10.17504/protocols.io.4r3l22zbl1y/v1>) for microglia differentiation protocol for cell generation and media composition.

## Protocol Overview

- A. Microglial precursor validation
- B. Mature microglia validation

## General Notes

A list of reagents and relevant vendor information can be found in the table listed under the materials tab.

## Attachments



Microglia validation...

41KB

## Materials

















### Reagent Table:

Item	Vendor	Catalog Number
1xPBS	Corning	MT21031CV
Accutase	Gemini	400-158
EDTA	Fisher	BP120500
Trypan blue	Gibco	15250061
Human TruStain FcX	Biolegend	422302
APC Anti-human CD16	Biolegend	302102
APC Anti-human CD45	Biolegend	304012
APC anti-human CX3CR1	Biolegend	341610
PE Anti-human P2ry12	Biolegend	392103
PE Anti-human CD11b	Biolegend	301306
PE Anti-human CD14	Fisher	12014942
Calcein violet	Invitrogen	C34858



## Microglial precursor validation
















52m

- 1 Harvest microglial precursors from flasks in STEP4 by collecting the media into 15 ml conical tubes and centrifuging it at  150 rcf for  00:04:00 . 
- 2 Remove the supernatant and resuspend the cells in STEP4 media.
- 3 Count the cells and aliquot 100,000 cells/experiment condition in 15ml conical tubes.
- 4 Spin down the cells (  150 rcf for  00:04:00 ). 4m
- 5 Remove the supernatant and resuspend the cells in  100  $\mu\text{L}$  FACS buffer (PBS1x, EDTA 5 mM, 0.5% FBS).
- 6 Add  5  $\mu\text{L}$  /tube of human TruStain FcX and incubate for  00:10:00 at  Room temperature . 10m
- 7 Add primary antibodies for the desired experimental conditions (  1  $\mu\text{L}$  /tube for the antibodies described above or according to manufacturer's recommendations).
- 8 Incubate for  00:30:00 on  On ice . 30m
- 9 Wash the cells 2 times by adding  2 mL /tube of FACS buffer followed by centrifugation at  150 rcf for  00:04:00 4m
- 10 Resuspend the cells in  350  $\mu\text{L}$  of FACS buffer + calcein violet (follow manufacturer's instructions as to how to prepare calcein violet).
- 11 Keep your samples protected from light on ice and perform standart FACS analysis.







## Mature Microglia validation

32m

- 12 Remove the media from microglia cultures and add accutase (  1 mL /well in 6 well plates).
- 13 Incubate  00:10:00 at  37 °C . 10m
- 14 Add  3 mL of microglia maturation media to quench the reaction and collect the cells in 15 ml conical tubes.
- 15 Spin down at  150 rcf for  00:08:00 . 8m
- 16 Remove the supernatant and resuspend the cells in microglia maturation media.
- 17 Count the cells and aliquot 100,000 cells/experiment condition in 15ml conical tubes.
- 18 Spin down the cells (  150 rcf for  00:04:00 ). 4m
- 19 Remove the supernatant and resuspend the cells in  100 µL FACS buffer (PBS1x, EDTA 5 mM, 0.5% FBS).
- 20 Add  5 µL /tube of human TruStain FcX and incubate for  00:10:00 at  Room temperature . 10m
- 21 Add primary antibodies for the desired experimental conditions (  1 µL /tube for the antibodies described above or according to manufacturer's recommendations).
- 22 Incubate for  00:30:00  On ice . 30m



- 23 Wash the cells 2 times by adding with by adding  2 mL /tube of FACS buffer followed by centrifugation at  150 rcf for  00:04:00 .
- 24 Resuspend the cells in  350  $\mu$ L of FACS buffer + calcein violet (follow manufacturer's instructions as to how to prepare calcein violet).
- 25 Keep your samples protected from light on in ice and perform standard FACS analysis.

4m

## Protocol references

<https://doi.org/10.17504/protocols.io.4r3l22zbjl1y/v1>