JAN 30, 2024

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj3w2plk5/v1

Protocol Citation: mariangela.massarocenere, Valerio Chiurchiù 2024. Immunophenotyping of immune cells by high dimensional flow cytometry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj3w2plk5/v1

Protocol status: Working

Created: Jan 25, 2024

Immunophenotyping of immune cells by high dimensional flow cytometry

In 1 collection

mariangela.massarocenere[1,2,3], Valerio Chiurchiù[4,5]

[1]Department of Experimental Neuroscience, Santa Lucia Foundation IRCCS, Rome, Italy;
[2]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, United States;
[4]Laboratory of Resolution of Neuroinflammation, Santa Lucia Foundation IRCCS, Rome, Italy;
[5]Institute of Translational Pharmacology, National Research Council, Rome, Italy

n.berret n.berretta

ABSTRACT

Protocol to assess cellular phenotypes by multiparametric flow cytometry panels containing markers to identify cell types and activation states

Last Modified: Jan 30, 2024

PROTOCOL integer ID: 94139

Tissue dissociation

1 After animal sacrifice (see protocol “intracardiac perfusion”), the dissected and collected cerebral region of interest (SNpc and striatum) is immersed in D-PBS with high glucose buffer at 4°C

2 Immediately after, the brain regions are dissociated to single-cell suspension by enzymatic degradation and myelin removal using the adult brain dissociation kit and GentleMACS dissociator (Miltenyi Biotec.)

3 The isolated tissue are placed in C-tubes and a mix containing Enzyme P and Buffer Z is added

4 Add a second mix containing Enzyme A and Buffer Y, tightly close C-tubes and attach upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec.)

5 Run the appropriate program 37C_ABDK_02 for 30 min

6 Detach the C-tube and centrifugate to collect sample

7 Resuspend and filter to a 70 um MACS SmartStrainer with 10 ml of cold D-PBS with high glucose

8 After centrifugation at 300g for 10 minutes, resuspend the sample in the debris removal solution, gently overlay in cold D-PBS with high glucose and centrifuge at 3000g for 10 minutes with full acceleration brake.

9 Discard the two phases and resuspend the sample in cold D-PBS with high glucose

10 Centrifuge at 1000g for 10 minutes

11 Resuspend in the staining buffer (sample is ready for cell count and flow cytometry staining)

Immunophenotyping of brain tissues by flow cytometry

12 Stain single suspension cells with PerCP5.5-conjugated anti-CD45 (1:100, Biolegend), APC-Vio770-conjugated anti-CD11b/c (1:100, REAffinity Miltenyi Biotec.), APC-conjugated anti-CD161 (1:100, Biolegend) or CD86 (1:100, Miltenyi Biotec.), PE-conjugated anti-CD45RA (1:100, Biolegend), PE-Cy7-conjugated anti-CD3 (1:100, Miltenyi Biotec.) or anti-MHC-II (1:100, REAffinity Miltenyi Biotec.), and FITC-conjugated anti-granulocytes (1:100 Miltenyi Biotec.) or VioBright-conjugated anti-ACSA-2/O4/CD31 (1:100 REAffinity Miltenyi Biotec.) for 15 minutes at 4°C in the dark

13 Wash and resuspend in PBS

14 Acquire cells on an appropriate flow cytometer

15 Exclude astrocytes, oligodendrocytes, and endothelial cells based on their expression of ACSA-2, O4, and CD31 (Lineage). The remaining cells are identified as CD45+ total leucocytes/resident immune cells

16 Inside this gate, microglial cells are identified as CD45lowCD11b/c+ cells and infiltrated myeloid cells as CD45highCD11bhigh, subsequently identified as macrophages according to the expression of F4/80. The remaining of CD45highCD11blow cells are further gated to identify T-lymphocytes as CD3+, B-lymphocytes as CD45RA+, and NK cells as CD161+. The expression of CD68 and MHC-II is further assessed inside the microglial cell population

17 For each analysis, acquire at least 0.2x10^6 live by gating on aqua Live/Dead negative cells and then analyze using Flowjo software (Tree Star)