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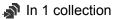
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(Immunophenotyping of immune cells by high dimensional flow cytometry



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ABSTRACT

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

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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

12 Stain single suspension cells with PerCP5.5-conjugated anti-CD45 (1:100, Biolegend), APC-Vio770conjugated 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 antigranulocytes (1:100 Miltenyi Biotec.) or VioBright-conjugated anti-ACSA-2/04/CD31 (1:100 REAffinity Miltenyi Biotec.) for 15 minutes at 4°C in the dark

Oct 30 2024

- 13 Wash and resuspend in PBS
- 14 Acquire cells on an appropriate flow cytometer

- 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
- 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 0.2x10⁶ live by gating on aqua Live/Dead negative cells and then analyze using Flowjo software (Tree Star)