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Protocol status: Working
We use this protocol and it's working

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Flow cytometry

Pranay Srivastava[1]

[1]Massachusetts General Hospital, ASAP

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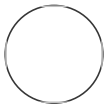

Fang Zhou
Massachusetts General Hospital, Aligning Science Across Park...

ABSTRACT

This protocol is to assess immune cell profile inn spleen (Chauhan et al. 2018).

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Keywords: ASAPCRN

1 Spleen was removed in a 35 mm petri plate with 5 ml RPMI 1640 and digested mechanically and passed through 70 µm filter screen.

2 The cell suspension was centrifuged, and the pellet was incubated in RBC lysis buffer. The resulting cell suspension was washed in 1xPBS and blocked with Fc Block (Biolegend, Cat# 101302, 1 µl/50 µl).

3 The cells were incubated with MC1R (Invitrogen, Cat# PIPA521911, 1.39 µg) antibody followed by fluorophore conjugated primary antibodies for extracellular markers (Biolegend Cat# 101235, CD11b-BV421(0.25 µg); Cat# 127641, Ly6G-BV-650 (0.25 µg); Cat# 128041, Ly6C-BV785 (0.125 µg); Cat# 100516, CD4-APC (0.25 µg); Cat# 100751, CD8a-BV510 (0.5 µg); Cat# 152405, CD19-PerCP-Cy5.5 (0.25 µg); Cat# 102036, CD25-BV605 (0.3 µg)) and AF488 (Invitrogen, Cat# A11034, 1:200).

4 Zombie dye (Biolegend, Cat# 423101, 1µl/sample) was used to differentiate between live and dead cells. Helper T cells and cytotoxic T cells were identified by CD4+ and CD8+, respectively. CD4+CD25+ cells were used to mark Tregs. CD19+ cells were used as marker for B cells. Monocytes were identified as CD11b+Ly6G-Ly6C^{high} cells and neutrophils were marked by CD11b+Ly6C-Ly6G+. For monocytes and neutrophils, CD11b-positive cells were first extracted from the live cell subset by expansion with SSC-A, followed by Ly6C. After expansion with Ly6C and Ly6G, we excluded Ly6G-positive cells. Monocytes were identified as CD11b+Ly6G-Ly6C high cells, and neutrophils were marked by CD11b+Ly6C-Ly6G+.

5 SORP 5 Laser BD Fortessa X-20 (BD Bioscience) and FlowJo v10.7.1 (Becton Dickson & Company) software was used for data acquisition.