

Extra- and intra-cellular staining protocol for flow cytometry
Ben Green, 2023

1) Plate cells

- b. Plate 200µL of liver cells & 150µL of spleen cells in 96 well plate
- b. Centrifuge 400g, 5 min, 4°C
- c. Decant supernatant

2) Zombie - 20 min in dark on ice

- a. Add 1000µL PBS to 4 µL Zombie
- c. Add 50µL/well
- d. Incubate 20 min in dark
- e. Quench with 150 µL FACS Buffer
- f. Centrifuge 400g, 5 min, 4°C
- g. Decant supernatant

3) Fc Block - 10 min in dark on ice

[1:50 ratio of Fc Block diluted in FACS Buffer]

- a. Add 50µL Fc Block/well
- b. Incubate 10 min in dark
- c. Quench with 150µL FACS Buffer
- d. Centrifuge 400g, 5 min, 4°C
- e. Decant supernatant

4) Surface antibody master mix - 30 min in dark on ice

- b. Add total volume of FACS Buffer to tray, then surface antibody stains to FACS trough
- d. Mix stains into FACS using 1000µL pipette
- e. Add 50µL master mix/well
- f. Incubate 30 min in dark
- g. Quench with 150µL FACS Buffer
- h. Centrifuge 400g, 5 min, 4°C
- i. Decant supernatant

5) Fix Perm (Transcription Factor Solution) - 45 min in dark on ice

- a. Resuspend pellets in 100µL 1x Fix Perm Buffer
- b. Incubate ≥ 45 min in dark on ice
- c. Wash with 100µL 1x Perm-Wash Buffer
- d. Centrifuge 400g, 5 min, 4°C
- e. Decant supernatant

6) Intracellular antibody mix – 30 min in dark on ice

- a. Add 50µL intracellular antibody mix (diluted in perm-wash)
- b. Incubate 30 min in dark on ice
- c. Quench with 150µL FACS Buffer
- d. Centrifuge 400g, 5 min, 4°C
- e. Decant supernatant

7) Resuspend pellet in 200µL FACS Buffer, then flow