SEP 18, 2023

OPEN ACCESS

# JHM-MSMP Muscle Flow Cytometry

ccherry[1]

[1]JHM-MSMP Muscle Flow Cytometry

SenNet JMN-MSMP

ccherry

ABSTRACT

Muscle flow cytometry

**Protocol Citation:** ccherry 2023. JHM-MSMP Muscle Flow Cytometry. **protocols.io** https://protocols.io/view/jhm-msmp-muscle-flow-cytometry-cz4qx8vw



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

**Created:** Sep 18, 2023

**Last Modified:** Sep 18, 2023

**PROTOCOL integer ID:** 87920

## Protocol

1 Prepare viability dye solution for 100 µL / well to be stained. Dilute stock dyes in PBS.

2 Spin down plate at 400g for 5 min at 4°C or room temp.

3 Resuspend cells in 100µL of appropriate viability dye solution or 100µL of PBS for Unstained samples, all non-viability single-color controls (SCCs), and the viability fluorescence minus one (FMO) control.

4 Incubate cells in viability solution for 30 min on ice. Cover plate with foil to protect samples from light.

5 During incubation, make the antibody cocktails for the “all stain” master mix (to stain samples and all-stained control) as well as any FMOs or SCCs you may be running. FMOs & SCCs can be prepared in 8-tube PCR strips. All antibody mixes should be prepared in a concentrated stain buffer: Fc Block (BioLegend) + Monocyte Block (BioLegend) + Brilliant Stain Buffer (BD Biosciences). Then just before staining cells, use flow wash buffer to bring all stain mixes to correct volume to stain all samples and controls. Flow wash buffer: 1X PBS w/o $Ca^{2+}$ or $Mg^{2+}$ (Gibco 14190-144), 1% w/v bovine serum albumin (Sigma A9647), 1 mM EDTA pH 8.0 (Invitrogen 15575-038). A suggested panel for staining stromal, immune, and vascular cells is included:

| Fluorophores | Marker | Dilution |
|---|---|---|
| BV421 | CD31 | 1:400 |
| BV605 | CD45 | 1:150 |
| BV711 | CD11b | 1:300 |
| | | |
| AF488 | CD3 | 1:200 |
| | | |
| tdTomato | p16 | N/A |
| PE-Cy7 | F4/80 | 1:250 |
| | | |
| AF647 | CD146 | 1:2000 |
| AF700 | CD29 | 1:100 |
| eFlour780 | Viability | 1:1000 |

6 Following incubation in viability buffer, wash cells twice using flow wash buffer. Centrifuge at 400g for 5 minutes at 4°C or room temp. between each wash.

7 After final wash, resuspend cells in 100µL of the “All Stain” master mix or in the appropriate volume of the FMO & SCC antibody cocktails.

8 Incubate cells in antibody staining cocktail on ice for 45 min. Cover plate with foil to protect samples from light.

9 Following incubation, wash cells twice, as before, using flow wash buffer.

10 After 2nd wash, resuspend all samples and controls in 100µL of FluoroFix Buffer (BioLegend). Incubate for 15 minutes at room temperature. Cover plate with foil to protect samples from light. Note: If performing cell sorting, do not fix cells. Proceed instead with standard protocols for your specific cell sorter.

11 Wash cells twice after incubation using flow wash buffer.

12 After final wash, resuspend samples and controls in the 200µL volume of flow wash buffer. Cover the plate with foil and store at 4°C overnight.

13 Before acquiring samples on the Attune NXT cytometer, centrifuge and resuspend them in the appropriate volume of PBS.

14 Analyze samples on Attune NXT cytometer. Voltages for FSC, SSC, and all dyes should be set using unstained and single-color controls (SCCs). Tip: The tdTomato signal in the mouse quadriceps is bright, and the voltage will likely need to be turned down to prevent the signal from going off-scale.