DEC 15, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldm688l5b/v1

Protocol Citation: rebeccawallings 2023. Patient PBMC flow cytometry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldm688l5b/v1

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

Created: Dec 14, 2023

Last Modified: Dec 15, 2023

Patient PBMC flow cytometry

rebeccawallings[1]

[1]University of Florida

rebeccawallings

ABSTRACT

Patient PBMC flow cytometry

PROTOCOL integer ID:
92335

Funders Acknowledgement:
ASAP
Grant ID: ASAP-020527

1 1 x 10^6 PBMCs were taken for flow cytometry and transferred to a v-bottom 96-well plate (Sigma, CLS3896-48EA) and centrifuged at 300*x g* for 5 minutes at 4°C.

Cells were resuspended in 50 µL of PBS containing diluted fluorophore-conjugated antibodies and incubated in the dark at 4°C for 20 minutes. Cells were centrifuged at 300*x g* for 5 minutes at 4°C and washed in PBS x 2. Cells were fixed in 50 µL of 1% paraformaldehyde (PFA) at 4°C in the dark for 30 minutes.

Cells were centrifuged at 300*x g* for 5 minutes and resuspended in 200 µL FACs buffer (PBS, 0.5 mM EDTA, 0.1% sodium azide).

Cells were taken for flow cytometry on a MACS Quant Analyzer (Miltenyi). A minimum of 100,000 events were captured per sample and data were analyzed using FlowJo version 10.6.2 software (BD Biosciences).

When validating flow cytometry panels and antibodies, fluorescence minus one controls (FMOCs) were used to set gates and isotype controls were used to ensure antibody-specific binding.