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Patient PBMC flow cytometry

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We use this protocol and it's working

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Abstract

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Troubleshooting

1 1×10^6 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.