

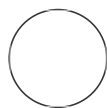


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## 🌐 Patient PBMC flow cytometry

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ABSTRACT

Patient PBMC flow cytometry

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

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**Funders**

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- 1  $1 \times 10^6$  PBMCs were taken for flow cytometry and transferred to a v-bottom 96-well plate (Sigma, CLS3896-48EA) and centrifuged at  $300\times g$  for 5 minutes at  $4^{\circ}\text{C}$ .

Cells were resuspended in 50  $\mu\text{L}$  of PBS containing diluted fluorophore-conjugated antibodies and incubated in the dark at  $4^{\circ}\text{C}$  for 20 minutes. Cells were centrifuged at  $300\times g$  for 5 minutes at  $4^{\circ}\text{C}$  and washed in PBS  $\times 2$ . Cells were fixed in 50  $\mu\text{L}$  of 1% paraformaldehyde (PFA) at  $4^{\circ}\text{C}$  in the dark for 30 minutes.

Cells were centrifuged at  $300\times g$  for 5 minutes and resuspended in 200  $\mu\text{L}$  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 (FMOs) were used to set gates and isotype controls were used to ensure antibody-specific binding.