


Sep 01, 2022

Time of flight mass cytometry (CyTOF)

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1 Works for me

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ABSTRACT

Staining procedure for cells analyzed by time of flight mass cytometry.

DOI

dx.doi.org/10.17504/protocols.io.5qpvo522dl4o/v1

PROTOCOL CITATION

Peter Hinderlie, Philippa R Kennedy 2022. Time of flight mass cytometry (CyTOF). **protocols.io**
<https://protocols.io/view/time-of-flight-mass-cytometry-cytof-bff8jjrw>



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CREATED

Apr 22, 2020

LAST MODIFIED

Sep 01, 2022

PROTOCOL INTEGER ID

36064

- 1 Cells from separate donors or conditions are stained separately with Cisplatin (Cat. No. 201064, Fluidigm) followed by barcoding using the Cell-ID 20-Plex Pd Barcoding Kit (Cat. No. 201060, Fluidigm).
- 2 Following barcoding, samples are combined into a single 5 mL polystyrene U-bottom tube and incubated in the surface marker antibody cocktail for 30 min at 4 °C

- 3 Cells are then fixed using 2% PFA, for 20mins.
- 4 For intracellular staining, cells are permeabilized by incubation with Triton X 0.1% for 5 min at room temperature, followed by incubation with intracellular antibody cocktail for 30 min at 4 °C.
- 5 Stained cells are incubated overnight with Cell-ID Intercalator (Cat. No. 201192A, Fluidigm).
- 6 The following morning, cells are washed in either Maxpar PBS (Cat. No. 201058, Fluidigm), Maxpar Cell Staining Buffer (Cat. No. 201068, Fluidigm), or Millipore Water, with a spin at 1600 RPM for 4 min to remove the supernatant and replace it with fresh buffer.
- 7 Samples are run on a time of flight mass cytometer (CyTOF 2, Fluidigm) and analyzed with Cytobank (Cytobank Inc., RRID:SCR_014043) and Astrolabe Diagnostics (<https://astrolabediagnostics.com/>)