# Flow Protocol

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2024-10-24

## CD20 Flow Protocol

### Overview

- Prior to starting: Make cell stain
  - $-500\mu L PBS + 2\mu L Stain$
  - $-100\mu$ L of stain used/condition
- Cell stain made: 2000  $\mu L$  and 8  $\mu L$

#### **Protocol**

- 1. Resuspend cells and transferred to 3 wells of a 96 well plate
- 2. Spun down at 1500g for 2min
- 3. Flick media out
- 4. Resuspend w/  $100\mu$ L stain in well and consolidate in 1 well
- Add  $100\mu L$  to bottom well and mix until cells resuspended
- Take  $100\mu L$  CS-stain mix and add to well below and mix
- Repeat for third well and add the total CS-Stain mix to top well
- 5. Incubate in fridge for 25min
- In the dark
- 6. Spin down at 1500g for 2min
- 7. Flick out media
- 8. Resuspend in PBS and add to FACS tube
- Add an additional volume of PBS to dilute cells appropriately for flow
- Usually make it up to about  $300\text{-}400\mu\text{L}$  CS-PBS in the tube