Microglia FACS staining after isolation (from UCSD)

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This protocol details about microglia FACS staining after isolation (from UCSD).

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Microglia FACS staining, antibodies (CD11b, CD45)

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Gating Notes

- live vs dead
- FSC vs SSC
- singles vs. doublets.
- CD11B (high) vs. CD45 (low).
- CX3CR1 (optional) ◊ gate on positive cells.

Misc. Notes

- **don't need to compensate with only 2-3 colors.
- CD45, CD11B, and CX3CR1 are all surface markers.
- Usually get 20-50% live cells after staining.
- Better to do staining right after microglia prep rather than waiting O/N for staining.

Processing Notes

Can do RNA seq or Atac Seq

RNA Seq

- Spin down sample in Eppendorf (not in facs tubes
- Remove supernatant
- Add **150 µL** of trizol → resuspend
- Store in **-80 °C** (can keep stored for months).

ATAC Seq

- Do transposase reaction and then freeze for processing.

Materials

use 5ml Polyproprylene eppendorf tubes

Antibodies

- CD11b Monoclonal Antibody (M1/70)
  APC **eBioscience/Invitrogen Catalog #17-0112-82**

  Alexa Fluor® 488 anti-mouse CD45
- Antibody **BioLegend Catalog #103122**

  CD16/CD32 Monoclonal Antibody
- (93) **eBioscience/Invitrogen Catalog #14-0161-82**
- DAPI 1:10,000, for 45 sec, wash twice with PBS; dilute in H2O

Protocol 25m 45s

1 Resuspend cells staining buffer (**300 µL** of HBSS+EDTA+BSA).

- Resuspend in a 15-ml falcon tube.

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2 15m

Add Fc Block (~1:100 dilution) and incubate for **00:15:00** at **4 °C** or in the fridge.

- Add this to cells resuspended in the HBSS.

3 Take ~5% of cells for unstained control, put **On ice** .

Already did this during the isolation during the percoll separation step.

4

Add antibodies (CD11b, CD45, 1:100 dilution) for 20-30 min at **4 °C** .

4.1

Add directly to cells in the FC block.

4.2

Mix by tapping tube; do not vortex.

Don't add to unstained cells!!

5 45s

Add DAPI, **00:00:45** , then dilute with HBSS+BSA+EDTA.

5.1 Add 1:1000 dilution.

5.2 Add to unstained cells.

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6 Put 70-µm filter on top of a FACS test tube and filter resuspended pellet into the tube.

Do this for unstained samples as well.

6.1 Push filter hard onto tube.

7  10m

Centrifuge for **400 x g, 00:10:00** .

7.1 Prepare collection tubes during spin time.
- Coat FACS tubes with **1 mL** of staining buffer to prevent cells from sticking to walls of tubes.
- Invert tube several times.

8 Vacuum out supernatant.
- Remove most of supernatant; not all.

9 Resuspend pellet in **500 µL** to **1000 µL** staining buffer.

Depending on machine.

Keep cells on ice the whole time.

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