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Microglia FACS staining after isolation (from UCSD)

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

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Abstract

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

Attachments

437-925.docx
101KB

Guidelines

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

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.

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

- Add this to cells resuspended in the HBSS.

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

> Note
>
> 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.

> Note
>
> Don't add to unstained cells!!

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

5.1 Add 1:1000 dilution.

5.2 Add to unstained cells.

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

Note

Do this for unstained samples as well.

6.1 Push filter hard onto tube.

7 Centrifuge for 400 x g, 00:10:00 . 10m

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.

Note

Depending on machine.

Keep cells on ice the whole time.