

## **Reagent table:**

<b>Item</b>	<b>Vendor</b>	<b>Catalog #</b>
1xPBS	Corning	MT21031CV
Accutase	Gemini	400-158
EDTA	Fisher	BP120500
Trypan blue	Gibco	15250061
Human TruStain FcX	Biolegend	422302
APC Anti-human CD16	Biolegend	302102
APC Anti-human CD45	Biolegend	304012
APC anti-human CX3CR1	Biolegend	341610
PE Anti-human P2ry12	Biolegend	392103
PE Anti-human CD11b	Biolegend	301306
PE Anti-human CD14	Fisher	12014942
Calcein violet	Invitrogen	C34858

## **Protocol:**

Note: Please refer to microglia differentiation protocol (<https://doi.org/10.17504/protocols.io.4r3l22zbi1y/v1>) for cell generation and media composition.

## **Microglial precursor validation**

1. Harvest microglial precursors from flasks in STEP4 by collecting the media into 15 ml conical tubes and centrifuging it at 150 rcf/g - 4 min.
2. Remove the supernatant and resuspend the cells in STEP4 media.
3. Count the cells and aliquot 100k cells/experiment condition in 15ml conical tubes.
4. Spin down the cells (150 rcf/g - 4 min).
5. Remove the supernatant and resuspend the cells in 100 ul FACS buffer (PBS1x, EDTA 5 mM, 0.5% FBS).
6. Add 5 ul/tube of human TruStain FcX and incubate for 10 min at room temperature.
7. Add primary antibodies for the desired experimental conditions (1 ul/tube for the antibodies described above or according to manufacturer's recommendations).
8. Incubate for 30 min on ice.
9. Wash the cells 2 times by adding 2 ml/tube of FACS buffer followed by centrifugation at 150 rcf/g - 4 min.
10. Resuspend the cells in 350ul of FACS buffer + calcein violet (follow manufacturer's instructions as to how to prepare calcein violet).
11. Keep your samples protected from light on ice and perform standart FACS analysis.

### **Mature Microglia validation**

1. Remove the media from microglia cultures and add accutase (1 ml/well in 6 well plates).
2. Incubate 10 min at 37 C.
3. Add 3 ml of microglia maturation media to quench the reaction and collect the cells in 15 ml conical tubes.
4. Spin down at 150 rcf/g - 8 min.
5. Remove the supernatant and resuspend the cells in microglia maturation media.
6. Count the cells and aliquot 100k cells/experiment condition in 15ml conical tubes.
7. Spin down the cells (150 rcf/g - 4 min).
8. Remove the supernatant and resuspend the cells in 100 ul FACS buffer (PBS1x, EDTA 5 mM, 0.5% FBS).
9. Add 5 ul/tube of human TruStain FcX and incubate for 10 min at room temperature.
10. Add primary antibodies for the desired experimental conditions (1 ul/tube for the antibodies described above or according to manufacturer's recommendations).
11. Incubate for 30 min on ice.
12. Wash the cells 2 times by adding with by adding 2 ml/tube of FACS buffer followed by centrifugation at 150 rcf/g - 4 min.
13. Resuspend the cells in 350ul of FACS buffer + calcein violet (follow manufacturer's instructions as to how to prepare calcein violet).
14. Keep your samples protected from light on in ice and perform standard FACS analysis.