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Differential memory enrichment of cytotoxic CD4 T cells in Parkinson's disease patients reactive to α-synuclein - FACS of CELR2 expressing or non-expressing cells

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Antoine Freuchet[1], Emil Johansson[1], cecilia[1], Alessandro Sette[1]

[1]La Jolla Institute for Immunology

Emil Johansson
La Jolla Institute for Immunology

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

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Abstract

The purpose of this protocol is to sort CD4 Tem cells either expressing or not expressing CELSR2, to allow subsequent scRNAseq analysis.

Differential memory enrichment of cytotoxic CD4 T cells in Parkinson's disease patients reactive to α-synuclein - FACS sorting

1 Setup

Thaw vial(s) in waterbath for ~1min30sec
Transfer into warm 10mL HR5 + 20uL of Benzonase. Centrifuge 400g, 5min, RT
Resuspend in 10mL of PBS-BSA 0.04% and count. Centrifuge 400g, 5min, RT
Resuspend at 20.10^6 cells/mL in PBS-BSA 1% (chilled). Add 1 ml

2 Staining

1. Centrifuge 400g, 5min, RT.
2. incubate cells with Fc blocking solution (90 µl PBS and 10 µl FC block) for 10 min at 4C.
3. Centrifuge 400g, 5min, RT.
4. Resuspend cells in staining solution. ubcubate for 30 in at 4C in the dark

	Antibody target	Flurochrome	Amount per well (100 µl per well)
	CD3	AF700	2.5
	CD45RA	PE-Cy7	2.5
	CD8a	BV650	1.25
	CCR7	PerCP-Cy5.5	1.25
	CD4	BV711	1
	CELSR2	Purified	0.25
	HASHTAG	-	1
	BV Buffer	-	10
	PBS-BSA 1%	-	80.25

1. Centrifuge 400g, 5min, RT.
2. Wash three times in PBS-BSA 1%.
3. Resuspend in 2 mL secondary antibody mix (1992 µl PBS-BSA 1% and 8 µl PE-F(ab')2-Goat anti-Rabbit IgG (H+L) (A10542) per sample) and incubate for 30 min at 4C.
4. Wash three times in PBS-BSA 1%.
5. Resuspend in 2 mL of PBS-BSA 1%. Add DAPI prior to sorting to stain dead cells
6. Sort in 200 000 cells into PBS-FBS 20% pre-coated tubes