Apr 08, 2025

Differential memory enrichment of cytotoxic CD4 T cells in Parkinson's disease patients reactive to α-synuclein - CELSR2 staining protocol

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzk934vx1/v1

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

[1]La Jolla Institute for Immunology

Emil Johansson
La Jolla Institute for Immunology

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzk934vx1/v1

Protocol Citation: Antoine Freuchet, Emil Johansson, cecilia , Alessandro Sette 2025. Differential memory enrichment of cytotoxic CD4 T cells in Parkinson's disease patients reactive to α-synuclein - CELSR2 staining protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzk934vx1/v1

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

Created: February 10, 2025

Last Modified: April 08, 2025

Protocol Integer ID: 119902

Keywords: parkinson, expressing celsr2, differential memory enrichment, celsr2

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000375

Abstract

The purpose of this protocol is to quantify the number of CD4 T cells expressing CELSR2.

Prepare FACS buffer

1 **FACS buffer recipe (2mM EDTA, 2%FBS in PBS)**

1. 500ml PBS pH 7.4 Gibco
2. 10 ml FBS
3. 2ml 0.5 M EDTA
4. Filter through 0.2µm filter and de-gas for 30 minutes
5. Store at 4˚C

2 **Setup**

1. Thaw indicated number of vial(s) of PBMC.
2. Place the vials in 37C water bath for 1 min. Transfer to sterile 15 ml Falcon Tubes with 10 mL HR5 and 20 µL benzonase.
3. Centrifuge @ 1200 rpm (9/9 accel/break) for 7 min @ 4C.
4. Resuspend cells in 10 ml HR5 and count cell number.
5. Centrifuge cells @ 1200 rpm for 7 min @ 4C.
6. Resuspend HR5 medium to get a final concentration of 10×106 cells/ml.
7. Seed 100 µl of the 10×106 cells/ml cell suspension into 96U well plate (106 cells/100uL) for antibody staining.

3 **Antibody staining**

1. Centrifuge cells at 1400 rpm/5 min/4C.
2. Wash with 200 uL PBS per well and cfg at 1400 rpm/5 min/4C.]
3. Add 100µl/pts of Live/Dead eF506 (1/1000 in PBS) and Fc Block (1/80 in PBS). Incubate 20 min in dark at 4C.

	Reagent	Flurochrome	Amount per well (100 uL total)
	Viability Dye	eF506	0.1
	Fc Block	-	1.25
	PBS	-	98.65

1. Add 100uL of FACS Buffer/well. Centrifuge cells at 1400 rpm/5 min/4C.
2. Add 100uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
3. Resuspend cells in 50 uL of antibody mix and incubate for 30 min at 4C.

	Antibody target	Flurochrome	Amount per well (100 uL total)
	CD8a	BV650	1.25
	CD19	PE-Cy7	1.25
	CCR7	PerCP-Cy5.5	1.25
	CD45RA	eF450	2.5
	CD3	AF700	2.5
	CD4	BV711	1
	CELSR2	Unconjugated	0.5
	Brilliant stain buffer plus	-	10
	FACS Buffer	-	79.75

1. Add 100uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
2. Add 200uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
3. Add 200uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
4. Resuspend in 100 µL secondary antibody mix (99.6 µl FACS buffern and 0.4 µl PE-F(ab')2-Goat anti-Rabbit IgG (H+L) (A10542) per well) and incubate for 30 min at 4C.
5. Add 100uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
6. Add 200uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
7. Add 200uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
8. Resuspend in 150 µL of FACS Buffer /well.
9. Ready to acquire on flow cytometer