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

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We use this protocol and it's working

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Abstract

The purpose of this protocol is to quantify the number of cytotoxic CD4 T cells in the different memory compartments

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

Setup

2

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 20×106 cells/ml.
7. Seed 2×100 µl of the 20×106 cells/ml cell suspension into 96U well plate (20^6 cells/100uL) for each condition for the antibody staining.
8. For this experiment, seed two wells per donor for cells that will be stimulated with PMA/Ionomycin, and two wells that will be left unstimulated.
9. Add PMA and Ionomycin to reach a final concentration of 1µg/mL to the wells designated for stimulated cells.
10. Add GolgiPlug and GolgiStop to all wells, to reach a final volume of 2µg/mL. Incubate cells for 4h at 37°C 5% CO2.

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 100 µl/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

	Reagent	Flurochrome	Amount per well (100 uL total)
	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 100 uL of antibody mix and incubate for 30 min at 4C.

	Antibody target	Flurochrome	Amount per well (100 uL total)
	CD4	BUV395	2.50
	CD8	BV650	2.50
	CCR7	AF488	2.50
	CD45RA	BV570	2.50
	CD16	BUV737	2.50
	CD56	BV421	2.50
	CD94	PerCPCy5.5	2.50
	Brillian Stain Buffer Plus	-	10
	FACS Buffer	-	72.5

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. Fix and permeabilize cells by resuspending cells in 100 µl Fixation/Permeabilization working solution from the Intracellular Fixation & Permeabilization Buffer Set (ThermoFisher). Incubate cells for 30 minutes in the dark at room temperature (RT).
5. Resuspend cells in Intracellular antibody cocktail:

	Antibody target	Flurochrome	Amount per well (100 µl per well)
	CD3	AF700	2.50
	Perforin	PE-Cy7	2.50
	GzmB	PE-CF594	2.50
	Granulysin	PE	2.50

	Antibody target	Flurochrome	Amount per well (100 µl per well)
	CCL4	APC	2.50
	Brilliant Stain Buffer Plus	-	10
	1X Permeabilization Buffer	-	77.5

1. Incubate cells for 45 minutes at RT in the dark.
2. Add 100uL 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. Add 200uL of FACS Buffer /well. Centrifuge cells at 1400 rpm/5 min/4C.
5. Add 100 uL 4% PFA to each well. Incubate cells for 20 minutes in the dark at 4C.
6. Add 100uL 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