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PBMC- 04 - In vitro Culture of TEFF+TREG - Proliferation of TEFF

Marco Cosentino[1], Elisa Storelli[1], Alessandra Luini[1], Massimiliano LM Legnaro[1], Emanuela Rasini[1], Marco Ferrari[1], Franca Marino[1]

[1]Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)

1 Works for me dx.doi.org/10.17504/protocols.io.bkq7kvzn

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ABSTRACT

List of published work using this procedure:

- Kustrimovic, N., Comi, C., Magistrelli, L., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Minafra, B., Riboldazzi, G., Sturchio, A., Mauri, M., Bono, G., Marino, F., & Cosentino, M. (2018). Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. Journal of neuroinflammation, 15(1), 205. https://doi.org/10.1186/s12974-018-1248-8

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GUIDELINES

Work under laminar flow hood when you are processing samples, from the beginning to the end of the following procedure.

MATERIALS TEXT

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Fetal Bovine Serum

(FBS) **EuroClone Catalog #ECS0180L-500 ml**

RPMI

1640 **EuroClone Catalog #ECM 0495L- 500 ml**

Penicillin/Streptomycin **EuroClone Catalog #ECB3001D - 100 ml**

CPD-eFluor670 - 500

µg **eBioscience Catalog #65 0840 85**

L-Glutamine 100X -

100mL **EuroClone Catalog #ECB3000D**

M-Phytohaemagglutinin powder **Sigma**

Aldrich Catalog #L8902-25 mg

Human Interleukin 2 lyophilized powder research grade **Miltenyi**

Biotec Catalog #130-097-742

Instrumentation needed:

Sterile plastic disposables
Laminar Flow Hood
Humidified 37°C, 5% CO_2 incubator

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1 **Isolate TEFF and TREG** with Miltenyi Kit according to the **protocol PBMC- 03**.

2 **Count** both **TEFF** and **TREG** following the appropriate protocol. Leave TREG cells in their complete culture medium and proceed with TEFF cells.

3 **Stain TEFF with CPD** according to the appropriate protocol.

IMPORTANT!

It is necessary to have an initial number of **TEFF** of **at least 1x10^6 for staining**.

Include in your experiment also**TEFF cells unlabeled with CPD**, as fluorescent background control for FACS analysis (see the appropriate protocol in flow cytometry).

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4 Use sterile **96-well round bottom plates**.

These plates can contain a volume of maximum 250µL

5 Centrifuge TEFF and TREG at **1200 x g, Room temperature , 00:05:00**

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

6 Resuspend **TEFF** (CPD stained and unstained) and **TREG** in complete culture medium at a **concentration of 1x10^6/mL**.

7 According to the experimental design, **activate** a desired number of wells containing **TEFF cells** (CPD stained and unstained) with **PHA 5µg/ml** (final concentration) and **IL-2 40 ng/mL** (final concentration) by diluting the stock aliquots. Leave also wells of **TEFF** (CPD stained an dunstained) **unstimulated** (resting control).

8 Put **TEFF-CPD labeled cells** and **TREG cells** in the 96-well plate at a **ratio of 1:1** (for example, 0.1x10^{6}TEFF+0.1x10^{6}TREG) and activate the cells il the well directly (see step 7 for concentrations): include **1 control co-culture** (not treated with test substance) and **treated co-cultures** (+test substance) according to your experimental design.

9 Include also a culture of **resting** and **activated TEFF alone stained and unstained CPD** (for example 0.2x10^6 cells per well), as control for the subsequent flow cytometric analysis.

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10 Put the plate in a 37°C incubator for 120 hours.

11 At the end of cell culture, collect the cells in BD tubes and centrifuge them at

1200 x g, Room temperature , 00:05:00

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

12 Proceed with the FACS protocol for TEFF+TREG proliferation.

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