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PBMC- 05 - In Vitro Culture of TEFF+TREG - Cytokine Production by TEFF V.2

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

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Keywords: cytokine production by teff list, treg in drug, parkinson, cytokine production, journal of neuroinflammation, treg, neuroinflammation, functional th1 bias

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

Materials

MATERIALS

- Fetal Bovine Serum (FBS) **EuroClone Catalog #**ECS0180L-500 ml
- RPMI 1640 **EuroClone Catalog #**ECM 0495L- 500 ml
- Penicillin/Streptomycin **EuroClone Catalog #**ECB3001D - 100 ml
- L-Glutamine 100X - 100mL **EuroClone Catalog #**ECB3000D
- M-Phytohaemagglutinin powder **Merck MilliporeSigma (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

Troubleshooting

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

2 **Count** both **TEFF** and **TREG** following the appropriate protocol (**CELL COUNT- 02** or **CELL COUNT- 03**).

3 Use sterile 96-well round bottom plates.

Note

[Consider that these plates can contain a volume of maximum 250µL]

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

Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

5 Resuspend **TEFF** and **TREG** in **SOLUTION- 13** at a **concentration of 1×10^6/mL**.

Document

NAME

SOLUTION- 13 - Complete culture medium

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Preview

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

7 Put **TEFF-CPD labeled cells** and **TREG cells** in the 96-well plate at a **ratio of 1:1**(for example, 0.1×10^6 TEFF + 0.1×10^6 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.

8 Include also a culture of **resting** and **activated TEFF alone** (for example 0.2×10^6per well), as control for the subsequent ELISA test.

9 Put the plate in a 37 °C incubator for 48 hours.

10 At the end of cell culture, **collect the supernatant** in eppendorf tubes (1.5mL) and **centrifuge them** at 1200 x g, Room temperature, 00:05:00 in order to eliminate any residual cells.

Equipment	
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11 Collect **supernatants** and **store them at -80°C** until ELISA assay is performed.