Nov 19, 2020

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

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1 Works for me dx.doi.org/10.17504/protocols.io.bmhxk37n

Farmacologia Medica

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

DOI

dx.doi.org/10.17504/protocols.io.bmhxk37n

PROTOCOL CITATION

Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PBMC- 05 - In Vitro Culture of TEFF+TREG - Cytokine Production by TEFF. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bmhxk37n

CREATED

Sep 18, 2020

LAST MODIFIED

Nov 19, 2020

PROTOCOL INTEGER ID

42263

MATERIALS TEXT

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 **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.

3 Use sterile 96-well round bottom plates.

[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**

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

5 Resuspend **TEFF** and **TREG** in complete culture medium at a **concentration of 1x10^6/mL**.

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, 0.1x10^6 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.

8 Include also a culture of **resting** and **activated TEFF alone** (for example 0.2x10^{6}per 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.

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

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