



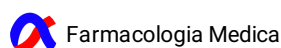
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PBMC- 05 - In Vitro Culture of TEFF+TREG - Cytokine Production by TEFF

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



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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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% CO₂ 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**

Allegra AVANTI 30
Centrifuge
Beckman Coulter Beckman Italy

- 5 Resuspend **TEFF** and **TREG** in complete culture medium at a **concentration of 1×10^6 /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, 0.1×10^6 TEFF + 0.1×10^6 TREG) and activate the cells in 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 0.2×10^6 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.

Allegra AVANTI 30
Centrifuge
Beckman Coulter Beckman Italy

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