



Version 2

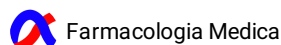
Nov 21, 2020

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

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1 Works for me [dx.doi.org/10.17504/protocols.io.bpxwmppe](https://dx.doi.org/10.17504/protocols.io.bpxwmppe)



## 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.bpxwmppe](https://dx.doi.org/10.17504/protocols.io.bpxwmppe)

## 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.

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<https://dx.doi.org/10.17504/protocols.io.bpxwmppe>

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## CREATED

Nov 21, 2020

## LAST MODIFIED

Nov 21, 2020

## PROTOCOL INTEGER ID

44758

## 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<sub>2</sub> incubator

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<https://doi.org/10.1186/s12974-018-1248-8>

- 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.

**[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 **SOLUTION- 13** at a **concentration of  $1 \times 10^6$ /mL**.



SOLUTION- 13 - Complete culture medium  
by Farmacologia Medica

- 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,  $0.1 \times 10^6$  TEFF +  $0.1 \times 10^6$  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  $0.2 \times 10^6$  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  
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- 11 Collect **supernatants** and **store them at -80°C** until ELISA assay is performed.