



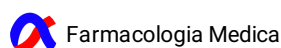
Nov 19, 2020

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

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1 Works for me [dx.doi.org/10.17504/protocols.io.bkq7kvzn](https://dx.doi.org/10.17504/protocols.io.bkq7kvzn)



## 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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41471

## GUIDELINES

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

## 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

 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<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. 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<sup>6</sup> 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).

- 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  $1 \times 10^6/\text{mL}$** .
- 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 and unstained) **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,  $0.1 \times 10^6 \text{ TEFF} + 0.1 \times 10^6 \text{ TREG}$ ) 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.
- 9 Include also a culture of **resting** and **activated TEFF alone stained and unstained CPD** (for example  $0.2 \times 10^6$  cells per well), as control for the subsequent flow cytometric analysis.

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