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Version 2

## ♥ PBMC- 04 - In vitro Culture of TEFF+TREG - Proliferation of TEFF V.2

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 44757

Keywords: treg in drug, parkinson, proliferation of teff list, treg, journal of neuroinflammation, neuroinflammation

#### **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. <a href="https://doi.org/10.1186/s12974-018-1248-8">https://doi.org/10.1186/s12974-018-1248-8</a>

#### **Guidelines**

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

#### **Materials**

#### **MATERIALS**

- X 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
- X 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:

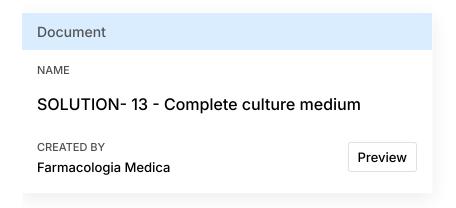
Sterile plastic disposables Laminar Flow Hood Humidified 37°C, 5% CO<sub>2</sub> incubator



# Troubleshooting



- 1 Isolate TEFF and TREG with Miltenyi Kit according to the <u>protocol PBMC- 03</u>.
- Count both TEFF and TREG following the appropriate protocol (CELL COUNT- 02, or CELL COUNT- 03). Leave TREG cells in their SOLUTION- 13 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 alsoTEFF 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**.

Note

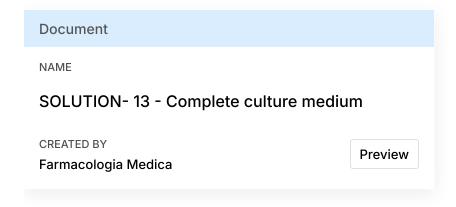
These plates can contain a volume of maximum 250µL

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



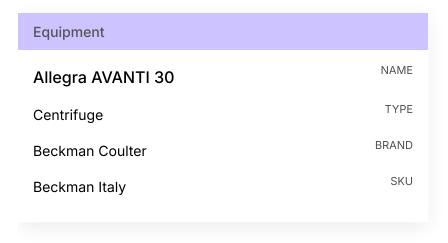


Resuspend **TEFF** (<u>CPD stained and unstained</u>) and **TREG** in **SOLUTION- 13** at a **concentration of 1×10**<sup>6</sup>/mL.



- According to the experimental design, **activate** a desired number of wells containing **TEFF cells** (<u>CPD stained and unstained</u>) with **PHA 5μg/ml** (final concentration) and **IL-2 40 ng/mL** (final concentration) by diluting the stock aliquots. Leave also wells of **TEFF** (<u>CPD stained and dunstained</u>) **unstimulated** (resting control).
- Put **TEFF-CPD labeled cells** and **TREG cells** in the 96-well plate at a **ratio of 1:1**(for example, 0.1×10<sup>6</sup>TEFF+0.1×10<sup>6</sup>TREG) and activate the cells if 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.

- Include also a culture of resting and activated TEFF alone stained and unstained 9 CPD (for example 0.2×10<sup>6</sup> 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 **3** 1200 x g, Room temperature, 00:05:00



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