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

DOI

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Marco Cosentino¹, Elisa Storelli¹, Alessandra Luini¹, Massimiliano LM Legnaro¹, Emanuela Rasini¹, Marco Ferrari¹, Franca Marino¹

¹Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)



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Protocol status: Working

We use this protocol and it's working

Created: November 21, 2020

Last Modified: November 21, 2020

Protocol Integer ID: 44757



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 Treq in drug-naïve and drug-treated patients. Journal of neuroinflammation, 15(1), 205. https://doi.org/10.1186/s12974-018-1248-8

Guidelines

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

Materials

MATERIALS

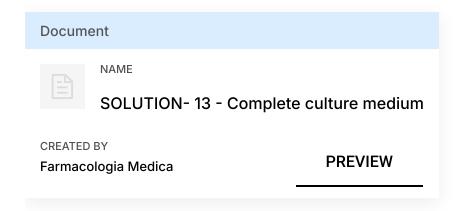
- Fetal Bovine Serum (FBS) EuroClone Catalog #ECS0180L-500 ml
- RPMI 1640 EuroClone Catalog #ECM 0495L- 500 ml
- Penicillin/Streptomycin EuroClone Catalog #ECB3001D 100 ml
- **Σ** CPD-eFluor670 500 μg **eBioscience Catalog** #65 0840 85
- M-Phytohaemagglutinin powder Sigma Aldrich Catalog #L8902-25 mg
- 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



- 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.

Note

IMPORTANT!

It is necessary to have an initial number of **TEFF** of **at least 1x10⁶ 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**.

Note

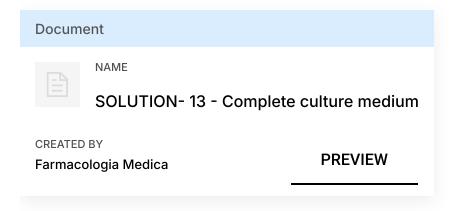
These plates can contain a volume of maximum 250µL

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



Equipment	
Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

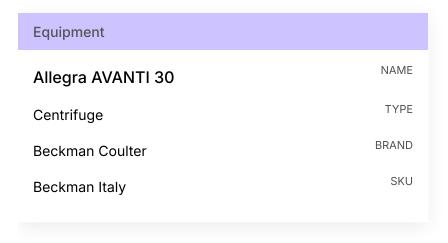
Resuspend **TEFF** (<u>CPD stained and unstained</u>) and **TREG** in **SOLUTION- 13** at a **concentration of 1×10**6/mL.



- 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, 0.1×10⁶TEFF+0.1×10⁶TREG) 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.



- Include also a culture of resting and activated TEFF alone stained and unstained 9 CPD (for example 0.2×10⁶ 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.