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Protocol status: Working
We use this protocol and it's working

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

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Farmacologia Medica

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>

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**
- ☒ 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% CO₂ incubator

- 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**). Leave TREG cells in their **SOLUTION- 13** and proceed with TEFF cells.

Document

NAME
SOLUTION- 13 - Complete culture medium

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- 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 1×10^6 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 250 μ L

- 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

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

Document

NAME

SOLUTION- 13 - Complete culture medium

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- 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, 0.1×10^6 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.
- 9 Include also a culture of **resting** and **activated TEFF alone stained and unstained CPD** (for example 0.2×10^6 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**

Equipment	
Allegra AVANTI 30	NAME
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12 Proceed with the FACS protocol for TEFF+TREG proliferation.