Version 2
Nov 21, 2020

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

Marco Cosentino[1], Elisa Storelli[1], Alessandra Luini[1], Massimiliano LM Legnaro[1], Emanuela Rasini[1], Marco Ferrari[1], Franca Marino[1]

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

1 Works for me dx.doi.org/10.17504/protocols.io.bpxvmpn6

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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.bpxvmpn6

PROTOCOL CITATION

Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PBMC- 04 - In vitro Culture of TEFF+TREG - Proliferation of TEFF. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpxvmpn6
Version created by Farmacologia Medica

CREATED

Nov 21, 2020

LAST MODIFIED

Nov 21, 2020

PROTOCOL INTEGER ID

44757

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_2 incubator

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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.

SOLUTION- 13 - Complete culture medium
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3 **Stain TEFF with CPD** according to the appropriate protocol.

IMPORTANT!

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It is necessary to have an initial number of **TEFF** of **at least 1x10^6 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**.

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 **SOLUTION- 13** at a **concentration of 1x10^6/mL**.

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 an dunstained) **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, 0.1x10^{6}TEFF+0.1x10^{6}TREG) 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.

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9 Include also a culture of **resting** and **activated TEFF alone stained and unstained CPD** (for example $0.2x10^6$ 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
Beckman Coulter **Beckman Italy**

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

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