Nov 21, 2020 Version 2

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

DOI
dx.doi.org/10.17504/protocols.io.bpxvmpn6

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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**

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 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% CO_2 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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PREVIEW

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 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**.

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

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

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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PREVIEW

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, 0.1×10^{6}TEFF+0.1×10^{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.

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
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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