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PMN- 02 - MTT Assay in Human PMN

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)

1 Works for me dx.doi.org/10.17504/protocols.io.bkq8kvzw

 Farmacologia Medica

ABSTRACT

Published work using this protocol:

- A Novel Standardized Cannabis sativa L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions. Mabou Tagne A, Marino F, Legnaro M, Luini A, Pacchetti B, Cosentino M. Int J Mol Sci. 2019 Apr 13;20(8):1833. doi: **10.3390/ijms20081833**

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MATERIALS

NAME	CATALOG #	VENDOR
Dimethyl sulfoxide (DMSO)	276855	Sigma Aldrich
Fetal Bovine Serum (FBS)	ECS0180L-500 ml	EuroClone
RPMI 1640	ECM 0495L- 500 ml	EuroClone
Thiazolyl blue tetrazolium bromide (MTT)	M5655	Sigma Aldrich
Penicillin/Streptomycin	ECB3001D - 100 ml	EuroClone

MATERIALS TEXT

Instrumentation needed:

Sterile and non sterile plastic disposables
ELISA plate reader





EQUIPMENT


EQUIPMENT

NAME	CATALOG #	VENDOR
Allegra AVANTI 30	Beckman Italy	Beckman Coulter

BEFORE STARTING





Use all reagents at Room Temperature


- 1 Isolate PMN according to the protocol PMN-01a for buffy coats or PMN-01b for fresh whole blood.
- 2 Resuspend PMN at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin
- 3 Add  **250 μ l** of cell suspension per well in a 96-well round bottom plate and incubate for  **24:00:00** alone or in the presence of the test substance at  **37 °C** in a 5% CO₂ atmosphere. **Cells must be plated in duplicate for each experimental condition.**
- 4 Centrifuge at  **1400 x g, 00:05:00** and discard the supernatant.



Allegra AVANTI 30
Centrifuge


Beckman Coulter Beckman Italy

- 5 Resuspend cells with  **200 μ l** of **MTT solution at 0.5 mg/ml**, incubate for  **02:00:00** at  **37 °C** in a 5% CO₂ atmosphere.
- 6 Centrifuge at  **1400 x g, 00:05:00** and discard the supernatant.



Allegra AVANTI 30
Centrifuge

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- 7 Add  **120 μ l** of **DMSO** per well, mix the plate until the formazan crystals are dissolved.
- 8 Read the plate on the ELISA plate reader at 570 nm.

