VERSION 1

SEP 17, 2020

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bkq8kvzw

Protocol Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PMN- 02 - MTT Assay in Human PMN. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bkq8kvzw>

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

Created: Sep 03, 2020

Last Modified: Sep 17, 2020

PROTOCOL integer ID:
41472

PMN- 02 - MTT Assay in Human PMN V.1

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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**

MATERIALS

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☒ Dimethyl sulfoxide (DMSO) **Sigma Aldrich Catalog #276855**

☒ Fetal Bovine Serum (FBS) **EuroClone Catalog #ECS0180L-500 ml**

☒ RPMI 1640 **EuroClone Catalog #ECM 0495L- 500 ml**

☒ Thiazolyl blue tetrazolium bromide (MTT) **Sigma Aldrich Catalog #M5655**

☒ Penicillin/Streptomycin **EuroClone Catalog #ECB3001D - 100 ml**

Instrumentation needed:

Sterile and non sterile plastic disposables

ELISA plate reader

BEFORE START INSTRUCTIONS

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.

Equipment

Allegra AVANTI 30

NAME

Centrifuge

TYPE

Beckman Coulter

BRAND

Beckman Italy

SKU

- 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.

Equipment	
Allegra AVANTI 30	NAME
Centrifuge	TYPE
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7 Add 120 µL 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.