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

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Protocol status: Working

We use this protocol and it's working

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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

MATERIALS

⊗ 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

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

Beckman Coulter

BRAND

Beckman Italy

SKU

- 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.