



Version 2

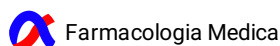
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## PMN- 02 - MTT Assay in Human PMN V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bpxzmpp6



### 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 TEXT

### 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

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### 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  $1 \times 10^6$  cells/ml in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin (**SOLUTION- 13**).



SOLUTION- 13 - Complete culture medium  
by **Farmacologia Medica**


- 3 Add  **250 µl** 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% CO<sub>2</sub> 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 µl** of **MTT solution at 0.5 mg/ml**, incubate for  **02:00:00** at  **37 °C** in a 5% CO<sub>2</sub> atmosphere.

6 Centrifuge at  **1400 x g, 00:05:00** and discard the supernatant.

Allegra AVANTI 30  
Centrifuge  
Beckman Coulter      Beckman Italy

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.