



Version 2

Nov 21, 2020

## PMN- 06 - Culture of Human PMN - TNF- $\alpha$ production V.2

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Works for me

[dx.doi.org/10.17504/protocols.io.bpx2mpqe](https://dx.doi.org/10.17504/protocols.io.bpx2mpqe)

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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**

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### PROTOCOL CITATION

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## MATERIALS TEXT

### MATERIALS

 Fetal Bovine Serum

(FBS) EuroClone Catalog #ECS0180L-500 ml

 RPMI

1640 EuroClone Catalog #ECM 0495L- 500 ml

 Lipopolysaccharides from Escherichia coli

0127:B8 Sigma Catalog # L3137

 Penicillin/Streptomycin EuroClone Catalog #ECB3001D - 100 ml

 ELISA kit for TNF-α Thermo Fisher

Scientific Catalog #KHC0031

 Cannabidiol Linnea

SA Catalog #not available

Instrumentation needed:

Sterile and non sterile plastic disposables  
ELISA plate reader

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- 1 Isolate PMN according to the protocol PMN-01a or PMN-01b.
- 2 Resuspend PMN at  $10 \times 10^6$  cells/ml in RPMI medium supplemented with 10% FBS and 1% penicillin/streptomycin (**SOLUTION- 13**).



SOLUTION- 13 - Complete culture medium  
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- 3 Add  **300 µl** of cell suspension in a 5 ml tube for cell culture and incubate for  **21:00:00** at  **37 °C** in 5% CO<sub>2</sub>.  
Cells were incubated in resting condition or in presence of stimulus. Stimulus (**LPS 1 µg/ml** and/or **CBD 10<sup>-8</sup>-10<sup>-5</sup> M**) in 5 ml tubes for cell culture. **Other possible activating stimuli** can be selected and included.
- 4 Centrifuge at  **1500 x g, 00:10:00**

Allegra AVANTI 30

Centrifuge

Beckman Coulter

Beckman Italy

- 5 Harvest cell-free supernatant (  150 µl x2 aliquots) and cell pellet.
- 6 Store the supernatant at -80°C until the TNF-α Enzyme Linked-Immuno-Sorbent Assay (ELISA) is performed.
- 7 Store cell pellet at -80°C for gene expression analysis (RT PCR).