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PMN- 06 - Culture of Human PMN - TNF- α production V.2

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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

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

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

⊗ Lipopolysaccharides from Escherichia coli O127: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



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

Document







NAME

SOLUTION- 13 - Complete culture medium

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PREVIEW

- 3 Add  300 μ L of cell suspension in a 5 ml tube for cell culture and incubate for  21:00:00 at  37 °C in 5% CO₂. Cells were incubated in resting condition or in presence of stimulus. Stimulus (**LPS 1 μ g/ml** and/or **CBD 10⁻⁸-10⁻⁵M**) 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



Equipment

Allegra AVANTI 30

NAME

Centrifuge


TYPE

Beckman Coulter

BRAND

Beckman Italy

SKU

- 5 Harvest cell-free supernatant ( 150 μ L 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).