VERSION 1

SEP 17, 2020

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bkrakv2e

Protocol Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PMN- 06 - Culture of Human PMN - TNF- α production. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bkrakv2e>

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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:
41474

PMN- 06 - Culture of Human PMN - TNF- α production V.1

Marco Cosentino¹, Elisa Storelli¹, Alessandra Luini¹, Massimiliano LM Legnaro¹, Emanuela Rasini¹, Marco Ferrari¹, Franca Marino¹

¹Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)

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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- ⊗ 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.
- 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^{-5} 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

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).