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PMN- 06 - Culture of Human PMN - TNF-α production

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1 Works for me dx.doi.org/10.17504/protocols.io.bkrakv2e

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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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MATERIALS

NAME	CATALOG #	VENDOR
Fetal Bovine Serum (FBS)	ECS0180L-500 ml	EuroClone
RPMI 1640	ECM 0495L- 500 ml	EuroClone
Lipopolysaccharides from Escherichia coli O127:B8	L3137	Sigma
Penicillin/Streptomycin	ECB3001D - 100 ml	EuroClone
ELISA kit for TNF-α	KHC0031	Thermo Fisher Scientific
Cannabidiol	not available	Linnea SA

MATERIALS TEXT

Instrumentation needed:

Sterile and non sterile plastic disposables

ELISA plate reader

EQUIPMENT

NAME	CATALOG #	VENDOR
Allegra AVANTI 30	Beckman Italy	Beckman Coulter

1 Isolate PMN according to the protocol PMN-01a or PMN-01b.

2 Resuspend PMN at 10x10^{6}cells/ml in RPMI medium supplemented with 10% FBS and 1% penicillin/streptomycin.

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_2.
Cells were incubated in resting condition or in presence of stimulus. Stimulus (**LPS 1 µg/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**

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).

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