



# PMN- 05 Culture of Human PMN - IL-8 production

Marco Cosentino<sup>1</sup>, Elisa Storelli<sup>1</sup>, Alessandra Luini<sup>1</sup>, Massimiliano LM Legnaro<sup>1</sup>, Emanuela Rasini<sup>1</sup>, Marco Ferrari<sup>1</sup>, Franca Marino<sup>1</sup>

<sup>1</sup>Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)

1 Works for me dx.doi.org/10.17504/protocols.io.bhdgj23w

Mattia Di Rocco

## ABSTRACT

### Adrenergic Modulation of Migration, CD11b and CD18 Expression, ROS and interleukin-8 Production by Human Polymorphonuclear Leukocytes.

Angela Scanzano, Laura Schembri, Emanuela Rasini, Alessandra Luini, Jessica Dallatorre, Massimiliano Legnaro, Raffaella Bombelli, Terenzio Congiu, Marco Cosentino, Franca Marino. Inflamm Res. 2015 Feb;64(2):127-35. doi: 10.1007/s00011-014-0791-8. Epub 2015 Jan 6.

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## MATERIALS

NAME	CATALOG #	VENDOR
RPMI 1640	ECM 0495L- 500 ml	EuroClone
Lipopolysaccharides from Escherichia coli O127:B8	L3137	Sigma
N-FORMYL-MET-LEU-PHE (fMLP)	F3506	Sigma Aldrich
ELISA Kit for IL-8	KHC0081	Invitrogen

## MATERIALS TEXT

### Instrumentation needed

Sterile and not sterile plastic disposable  
Laminar flow hood  
ELISA plate reader

## EQUIPMENT

NAME	CATALOG #	VENDOR
Allegra AVANTI 30	Beckman Italy	Beckman Coulter

- 1 Isolate PMN according either to the protocol "**Separation of Human Neutrophils (PMN) from Buffy CoatV.2**" or "**Separation of Human Neutrophils (PMN) from Whole Blood**".
- 2 Resuspend PMN at  $10 \times 10^6$  cells/ml in **RPMI medium**.
- 3 Use a number of tubes according to the experimental plan and to the substances to test.
- 4 Tubes will be prepared to test resting IL-8 production (substances alone) and stimulus-induced production.



Add substances before the activation with stimulus (time selected according to the experiential plan)  
Stimulus: fMLP 0.1  $\mu$ M or LPS 1  $\mu$ M or other.

- 5 Incubate PMN at  $37^\circ\text{C}$  for **03:00:00** in a 5 ml tubes for cell culture. Cells will be incubated in resting conditions or in presence of stimulus (point 3-4).
- 6 Centrifuge at **1500 x g 00:10:00**



Allegra AVANTI 30  
Centrifuge  
Beckman Coulter Beckman Italy

- 7 Harvest cell-free supernatant using a pipette and cell pellet (for real-time assay of mRNA for IL-8).
- 8 Store the supernatant and cell pellet at  $-80^\circ\text{C}$  until the IL-8 Enzyme Linked-ImmunoSorbent Assay (ELISA) and/or real time assay is performed.