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

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**Protocol status:** Working

We use this protocol and it's working

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## 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.



## Materials

### MATERIALS

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

⊗ Lipopolysaccharides from Escherichia coli O127:B8 **Sigma Catalog # L3137**

⊗ N-FORMYL-MET-LEU-PHE (fMLP) **Sigma Aldrich Catalog #F3506**

⊗ ELISA Kit for IL-8 **Invitrogen Catalog # KHC0081**

### Instrumentation needed

Sterile and not sterile plastic disposable

Laminar flow hood

ELISA plate reader



- 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.

#### Note

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

- 5 Incubate PMN at 37 °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

#### Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU



- 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°C until the IL-8 Enzyme Linked-ImmunoSorbent Assay (ELISA) and/or real time assay is performed.