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

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ABSTRACT

Published work using this protocol:

- 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 TEXT

MATERIALS

- RPMI 1640: EuroCloneCatalog #ECM 0495L- 500 ml
- Lipopolysaccharides from Escherichia coli O127:B8: SigmaCatalog # L3137
- N-FORMYL-MET-LEU-PHE (fMLP): Sigma AldrichCatalog #F3506
- ELISA Kit for IL-8: InvitrogenCatalog # KHC0081

Instrumentation needed

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

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- 1 Isolate PMN according either to the protocol "**PMN- 01a**" or "**PMN- 01b**".
- 2 Resuspend PMN at 10×10^6 cells/ml in complete culture medium (**SOLUTION- 13**).



SOLUTION- 13 - Complete culture medium
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- 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 μ M or LPS 1 μ 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^{3h} 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°C until the IL-8 Enzyme Linked-ImmunoSorbent Assay (ELISA) and/or real time assay is performed.