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PMN- 04 - Measurement of Intracellular Reactive Oxygen Species (ROS) levels in Human PMN

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Protocol status: Working

We use this protocol and it's working

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Abstract

List of published work using this protocol:

- Immune Profile of Hyaluronic Acid Hydrogel Polyethylene Glycol Crosslinked: An in Vitro Evaluation in Human Polymorphonuclear Leukocytes. Franca Marino, Marco Cosentino, Massimiliano Legnaro, Alessandra Luini, Julia Sigova, Roberto Mocchi, Trello Lotti, Nicola Zerbinati. *Dermatol Ther*. 2020 Apr 10; e13388. doi: 10.1111/dth.13388. Online ahead of print.
- A Novel Standardized Cannabis sativa L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions. Alex Mabou Tagne, Franca Marino, Massimiliano Legnaro, Alessandra Luini, Barbara Pacchetti, Marco Cosentino. *Int J Mol Sci*. 2019 Apr 13;20(8):1833. doi: 10.3390/ijms20081833.
- β 2 -Adrenoceptors Inhibit Neutrophil Extracellular Traps in Human Polymorphonuclear Leukocytes. Franca Marino, Angela Scanzano, Laura Pulze, Monica Pinoli, Emanuela Rasini, Alessandra Luini, Raffaella Bombelli, Massimiliano Legnaro, Magda de Eguileor, Marco Cosentino . *J Leukoc Biol*. 2018 Sep;104(3):603-614. doi: 10.1002/JLB.3A1017-398RR. Epub 2018 Apr 18.
- 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.
- The Essential Oil of Bergamot Stimulates Reactive Oxygen Species Production in Human Polymorphonuclear Leukocytes. Marco Cosentino, Alessandra Luini, Raffaella Bombelli, Maria T Corasaniti, Giacinto Bagetta, Franca Marino. *Phytother Res*. 2014 Aug;28(8):1232-9.doi: 10.1002/ptr.5121. Epub 2014 Jan 23.
- Immunomodulatory Properties of Achyrocline Satureioides (Lam.) D.C. Infusion: A Study on Human Leukocytes. Marco Cosentino, Raffaella Bombelli, Elena Carcano, Alessandra Luini, Franca Marino, Francesca Crema, Federico Dajas, Sergio Lecchini. . *J Ethnopharmacol*. 2008 Mar 28;116(3):501-7. doi: 10.1016/j.jep.2007.12.014. Epub 2008 Jan 6.



Materials

MATERIALS

⊗ NaCl **Sigma Aldrich Catalog #S9625**

⊗ C-DCFH-DA **Invitrogen - Thermo Fisher Catalog #C2938**

⊗ KCl **Sigma Catalog #P9541**

⊗ MgSO₄ **Sigma Catalog #1.05886**

⊗ Glucose **Sigma Catalog #1.08337**

⊗ CaCl₂ **Sigma Catalog #1.02382**

⊗ Hepes **Sigma Catalog #H7006**

Dubnoff bath



- 1 Isolate PMN using either protocol PMN- 01a or PMN 01b.
- 2 Suspend isolated PMN 1×10^6 cells/ml in **HBSS/HEPES medium**.

Document



NAME

SOLUTION- 12 – HBSS-HEPES for ROS measurement in PMN

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PREVIEW

- 3 Add 2×10^6 cells in 2 mL of **HBSS/HEPES** for each tube.
- 4 Incubate for 01:00:00 with 2 Micromolar (μM) of **C-DCFH-DA** in the **dark** at 37 °C (Dubnoff Bath).
- 5 Wash twice with 2 mL of **HBSS/HEPES**.

Each time centrifuge at 400 x g, Room temperature, 00:05:00

Document



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SOLUTION- 12 – HBSS-HEPES for ROS measurement in PMN

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PREVIEW

Equipment

Centrifuge

NAME

Eppendorf

BRAND

5804

SKU

6 Aspirate supernatant and resuspend with 2 mL of **HBSS/HEPES**.



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SOLUTION- 12 – HBSS-HEPES for ROS measurement in PMN

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PREVIEW

- 7 Recover PMN in cuvette of 2 ml in the **dark** at  37 °C (Dubnoff Bath).
- 8 Measurements of fluorescence must be performed by means of a **Spectrofluorimeter with excitation wavelength of 488 nm.**

Equipment

Spectrofluorimeter


NAME

Perkin Elmer

BRAND

LS50B

SKU

- 9 Cuvette must be maintained at  37 °C by means of a thermostatic bath, that will be turned on before the starting ROS measurement.
- 10 Fluorescence emission must be collected at **525 nm** and recorded in a specific data table that will be signed for each specific experimental plan (time for recording and complete time analysis), that will be signed by the researcher.
- 11 Intracellular ROS levels must be expressed as fluorescence intensity, in arbitrary units (AU) or according to the specific experimental plan.



