JUL 24, 2020

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

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Farmacologia Medica

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.biywkfxe

Protocol Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PMN- 04 - Measurement of Intracellular Reactive Oxygen Species (ROS) levels in Human PMN.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.biywkfxe>

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Protocol status: Working
We use this protocol and it's working

Created: Jul 24, 2020

Last Modified: Jul 24, 2020

PROTOCOL integer ID:
39670

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**.

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

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- 3 Add 2×10^6 cells in 2 mL of **HBSS/HEPES** for each tube.
- 4 Incubate for 01:00:00 with 2 Micromolar (μ M) 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

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Equipment

Centrifuge

NAME

Eppendorf

BRAND

5804

SKU

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

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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.