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PMN- 03 Culture of Human PMN - Migration

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1 Works for me dx.doi.org/10.17504/protocols.io.bhrmj546

Mattia Di Rocco

ABSTRACT

Published work using this protocol:

- 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.

DOI

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PROTOCOL CITATION

Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PMN- 03 Culture of Human PMN - Migration. **protocols.io** dx.doi.org/10.17504/protocols.io.bhrmj546

CREATED

Jun 22, 2020

LAST MODIFIED

Jul 07, 2020

PROTOCOL INTEGER ID

38413

MATERIALS

NAME	CATALOG #	VENDOR

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RPMI 1640	ECM 0495L- 500 ml	EuroClone
N-FORMYL-MET-LEU-PHE (fMLP)	F3506	Sigma Aldrich
Propanolol	P0884	Sigma Aldrich
Xylene	247642	Sigma-aldrich
Hematoxylin	MHS16	Sigma – Aldrich
IL-8	I1645	Sigma-aldrich

STEPS MATERIALS

NAME	CATALOG #	VENDOR
RPMI 1640	ECM 0495L- 500 ml	EuroClone
IL-8	I1645	Sigma-aldrich
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Propanolol	P0884	Sigma Aldrich
Hematoxylin	MHS16	Sigma – Aldrich
Xylene	247642	Sigma-aldrich

MATERIALS TEXT

Dubnoff bath

1 Separation of **PMN** (at least 7 million PMN).

2 **Preparation of 6 Boyden chambers**:
Cut the bottom part of the vial and apply the filter (with holes of 3-5 µm in diameter) with glue / mastic (fig. 1). With paper scotch, name all Boyden chambers (fig. 2).

figure 1

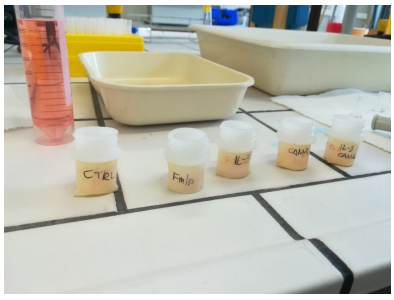

figure 2

3 Preparation of the solutions for:
- **IL-8** 10 ng/ml (2 µL stock in 198 µL di RPMI) chemotactic agent,
- **fMLP** 0.1 µM (10 µL stock in 990 µL di PBS/BSA) chemotactic agent,
- other activating stimulus according specific experimental plan,
- drug/substances to test at different concentrations: one chamber for each concentration.

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IL-8
by Sigma-aldrich
Catalog #: I1645

N-FORMYL-MET-LEU-PHE (fMLP)
by Sigma Aldrich
Catalog #: F3506

4 Fill the bottom of each Boyden chamber with **1 mL** of **RPMI**.

RPMI 1640
by EuroClone
Catalog #: ECM 0495L- 500 ml

5 Remove 10 µL of **RPMI** from each Boyden chamber and add **10 µl** of **IL-8** or **fMLP** treatment (except for control).

6 Resuspend 7x10^6 PMN in **700 µl** of **RPMI**.

7 Immerse the filters in the chambers.

8 Put **100 µl** of cell suspension in 4 vials and treat cells with **10 µl** of testing drug.

9 Place the cells on the filters (100 µL of cell suspension on each filter) using the P100 taking care to direct the tip towards the center of the filter without touching it (if the filter does not get wet at all, gently slam the chamber).

10 Cap and put Boyden's chambers in the Dubnoff bath for **01:30:00** .

11 Under the chemical hood, prepare the following material:
- 4 rows of mini becker (6 becker per row).

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In each row of becker arrange, in the following order, equal quantities (2 ml in total) of: **water**, **propanolol**, **xylene**, **propanolol + xylene** (fig. 3)

- 1 row of lids for 3-6 drops of hematoxylin to cover the same area of the filter (6 lids) and 5 plastic pasteur (each suitably marked with the name of the substance that will collect: **water**, **propanolol**, **xylene**, **hematoxylin**, immersion oil).(fig.4)

Figure 3

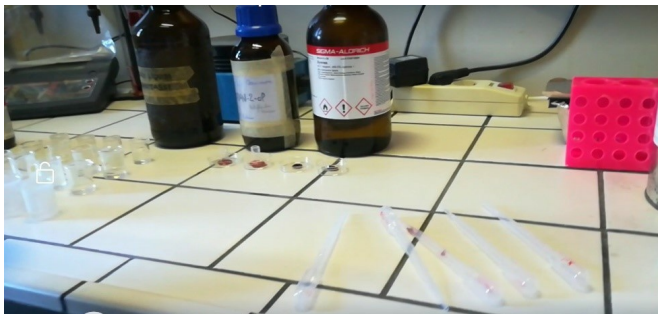

Figure 4

12 Immerse the filters related to eppendorf in:

- **PROPANOLOL** for **00:04:00**
- **WATER** for **00:04:00**
- **HEMATOXYLIN** for **00:06:00**
- **WATER** for **00:06:00**

(in the meantime throw the hematoxylin into the liquid discharge, wrap its containers in the paper and throw it all in special waste)

- **PROPANOLOL** for **00:06:00**
- **PROPANOLOL + XYLENE** for **00:06:00**

(meanwhile throw away water and propanolol in the liquid discharge and immerse the beakers to be washed in a bowl with water, alcohol and bleach taking care to turn them over so they are hygienized every side)

XYLENE for **00:06:00**

Propanolol
by Sigma Aldrich
Catalog #: P0884

Xylene
by Sigma-aldrich
Catalog #: 247642

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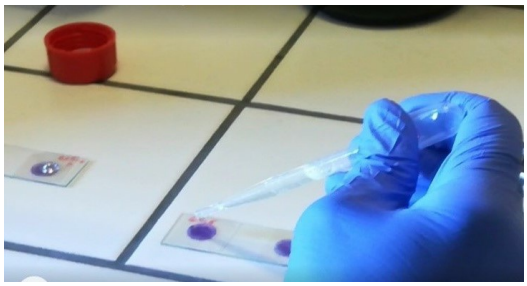
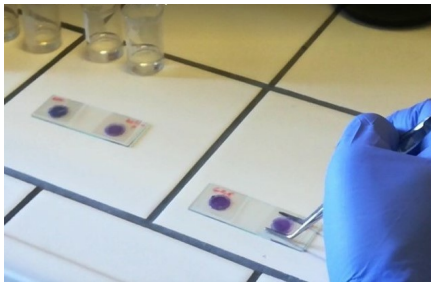
Hematoxylin
by Sigma – Aldrich
Catalog #: MHS16

13 Throw away the used pasteur, wrapping it first in the paper.

14 Set up the slides:
nominate them,
arrange the filter (taking care to maintain the correct orientation),
arrange the cover glass (by tapping laterally with the tweezers to make sure it is well stuck),
add on top a drop of immersion oil.





15

Microscopy: make sure that the lens comes into contact with the immersion oil.

16

Once the filter with the colored cells in Haematoxylin is in focus, using only the micrometric lens bring into focus the layer of neutrophils above and report the value by saying "UP"; then you go down into the depth of the filter (rotating the micrometric lens) and bring into focus the migrated neutrophil layer: mark the visual limit value of the individual migrated cells saying "DOWN".

17 The delta between the "UP" and "DOWN" values (express as µm) represents the distance in micrometers traveled by the migrating cells.

18 For each slide make 10 counts of the distance traveled by migrated neutrophils (selected in different optical fields): each count must be done by focusing on a different migrated neutrophil.

19 Results will be expressed or as absolute migration toward the filter or as ratio vs activating stimulus (sample migration/stimulus migration)

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