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PMN- 01b - Isolation of Human PMN from Whole Blood V.2

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

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ABSTRACT

List of published papers using this protocol:

- Boydum A.Isolation of mononuclear cells and granulocytes from human blood. Scand.J.Clin.Lab. Invest. 21 (Suppl.97): 77-89, 1968

- Alex Mabou Tagne, Franca Marino, Massimiliano Legnaro, Alessandra Luini, Barbara Pacchetti and Marco Cosentino. A Novel Standardized Cannabis sativa L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions. Int J Mol Sci2019 Apr; 20(8): 1833. Published online 2019 Apr 13. doi: 10.3390/ijms20081833.

- Angela Scanzano, Laura Schembri, Emanuela Rasini, Alessandra Luini, Jessica Dallatorre, Massimiliano Legnaro, Raffaella Bombelli, Terenzio Congiu, Marco Cosentino, Franca Marino. Adrenergic Modulation of Migration, CD11b and CD18 Expression, ROS and interleukin-8 Production by Human Polymorphonuclear Leukocytes. Inflamm Res. 2015 Feb;64(2):127-35. doi: 10.1007/s00011-014-0791-8. Epub 2015 Jan 6.

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PROTOCOL CITATION

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MATERIALS TEXT

MATERIALS

Ficoll Paque PLUS **Ge**

Healthcare Catalog #17144003-500 ml

Fetal Bovine Serum

(FBS) **EuroClone Catalog #ECS0180L-500 ml**

RPMI

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

NaCl **Sigma**

Aldrich Catalog #S9625

KHCO3 **Merck Serono**

GmbH Catalog #1.04854.500

EDTA **Sigma**

Aldrich Catalog #ED2SS

Acetic Acid 100% **Sigma**

Aldrich Catalog #A6283

Gentian violet 1% **Marco**

Viti Catalog #not available

NH4Cl **Merck Serono**

GmbH Catalog #1.01145.1000

Optical Microscope (for manual cell count)

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BEFORE STARTING

All reagents used in this protocol must be at room temperature

1 Place **5 mL** of whole blood into a 10 ml volume centrifuge tube.

2 Add **2 mL** of dextran solution **(SOLUTION- 03)** and mix well by drawing in and out of a pipette.

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SOLUTION- 03 - Dextran solution 5%
by Farmacologia Medica

3 Incubate in the **dark** for **00:30:00** at **37 °C**

4 Place **3 mL** of **Fycoll-HyPaque** media solution into a 10 ml volume centrifuge tube.

5 ⚠

Slowly and carefully layer the supernatant from blood/dextran mixture onto the Fycoll-HyPaque media solution.

Important: when layering the sample, do not mix the Fycoll-HyPaque media solution and supernatant.

6 Centrifuge at **400 x g, Room temperature , 00:30:00** with **no brake**.

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

7 Draw off the mononuclear cell layer at the Ficoll/plasma interface along with plasma and Ficoll media, leaving the white cell layer of granulocytes above the red blood cell layer undisturbed.

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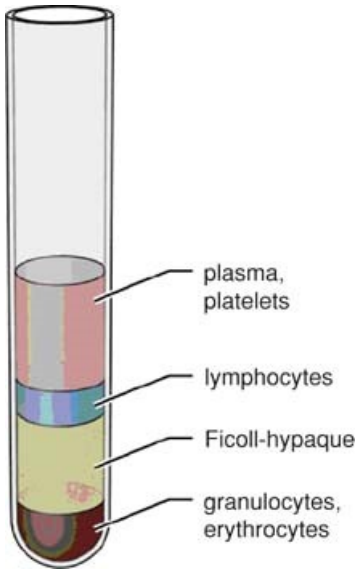

8 Resuspend the remaining cell layer in **5 µl** of NaCl 0.15 M (**SOLUTION- 01**) and centrifuge at **400 x g, Room temperature , 00:05:00**

SOLUTION- 01 - Sodium Chloride (NaCl) solution
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Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

9 Aspirate the supernatant with a plastic pipette Pasteur.

10 Lyse remaining red blood cells in **5 mL** of hypotonic Lysis Buffer (**SOLUTION- 06**) for **00:05:00**

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SOLUTION- 06 - Lysis Buffer
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

11 Centrifuge at **400 x g, Room temperature , 00:05:00**

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

12 Aspirate the supernatant with a plastic Pasteur pipette.

13 Resuspend the pellet in **5 mL** NaCl 0.15 M **(SOLUTION- 01).**

SOLUTION- 01 - Sodium Chloride (NaCl) solution
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14 Centrifuge at **400 x g, Room temperature , 00:05:00**

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

15 Aspirate the supernatant with a plastic Pasteur pipette.

16 Resuspend the cell pellet in **5 mL** NaCl 0.15 M **(SOLUOTIN- 01)** for manual cell counting using Türk solution (SOLUTION- 08). Follow protocol **CELL COUNT- 02**.

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SOLUTION- 01 - Sodium Chloride (NaCl) solution
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SOLUTION- 08 - Türk solution
by Farmacologia Medica

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OPTIONAL STEP

If needed, check the purity of PMN suspension by using morphological parameters of the flow cytometer.

For this test 0,5x10^6 PMN in **500 µl** of PBS (**SOLUTION- 02**) are enough.

SOLUTION- 02 - Phosphate Buffered Saline (PBS)
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BD FACSCelesta
Flow Cytometer
Becton Dickinson, Milan Italy **BD**

18 EXPECTED RESULTS

VIABILITY: the expeted viability by Trypan Blue shuold be ≥90%

CELL YIELD: ± 1 x10^6 cells starting from 1 mL of Whole Blood

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