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PMN- 01a - Isolation of Human PMN from Buffy Coat

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

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ABSTRACT

Separation of Human Neutrophils (PMN) from Buffy Coat: 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

- Ethylenediaminetetraacetic acid disodium salt dihydrate: Sigma AldrichCatalog #ED2SS
- Ficoll Paque PLUS: Ge HealthcareCatalog #17144003-500 ml
- Fetal Bovine Serum (FBS): EuroCloneCatalog #ECS0180L-500 ml
-RPMI 1640: EuroCloneCatalog #ECM 0495L- 500 ml
- NaCl: Sigma AldrichCatalog #S9625
- NH4Cl: Merck Serono GmbHCatalog #1.01145.1000
- KHCO3: Merck Serono GmbHCatalog #1.04854.500
- Acetic Acid 100%: Sigma AldrichCatalog #A6283
- Genitian violet 1%: Marco VitiCatalog #not available

Optical Microscope

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BEFORE STARTING

All reagents used in this protocol must be at room temperature

1 Place **5 mL** of venus blood from BUFFY COAT into 10 ml volume centrifuge tube.

2 Add **2 mL** of **SOLUTION- 03** and mix well drawing in and out of a pipette.

SOLUTION- 03 - Dextran solution 5%
by Farmacologia Medica

3 Incubate in the **DARK** for **00:45:00** at **37 °C**

45m

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

5 ⚠

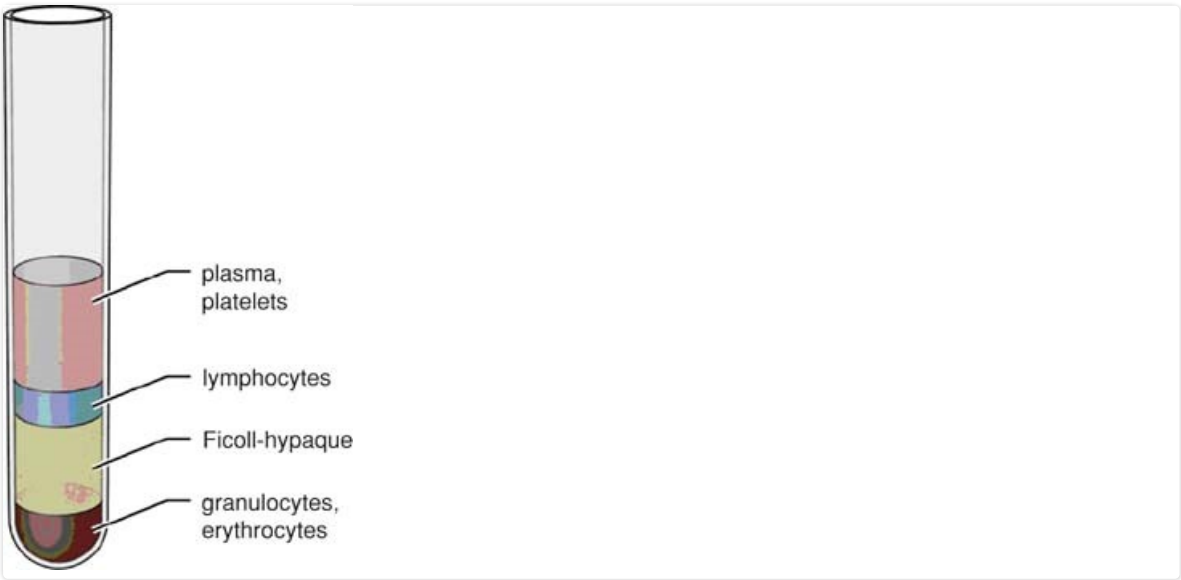
Slowly and carefully layer the supernatant from blood/dextran mixture onto the Fycoll-HyPaque media solution.

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

6 Centrifuge at **400 x g, Room temperature , 00:30:00** , no break

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.



8 Resuspend the remaining cell layer in **5 mL** of NaCl 0.15 Molarity (M) (**SOLUTION- 01**)and centrifuge at

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400 x g, Room temperature , 00:05:00

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

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

9 Aspirate the supernatant with a plastic Pasteur pipette.

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

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 Molarity (M) (**SOLUTION- 01**)

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

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 Molarity (M) (**SOLUTION- 01**)for manula cell counting with Türk solution (follow protocol CELL COUNT- 02).

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

SOLUTION- 08 - Türk solution
by Farmacologia Medica

17

OPTIONAL STEP

Cell viability can be checked using protocol CELL COUNT- 03 in the automated cell counter with Trypan Blue solution.

SOLUTION- 09 - Trypan Blue solution
by Farmacologia Medica

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Cellometer Auto T4
Automated Cell Counter
Nexcelom Bioscience **Euroclone**

18

OPTIONAL STEP

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

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

SOLUTION- 02 - Phosphate Buffered Saline (PBS)
by Farmacologia Medica

BD FACS Celesta
Flow Cytometer
Becton Dickinson **Milan Italy BD**

19 EXPECTED RESULTS

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

CELL YIELD: ± 6 x10^6 cells starting from 1 mL of Buffy Coat

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