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

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

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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.

Materials

- 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

Before start

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.

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SOLUTION- 03 - Dextran solution 5%

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

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.

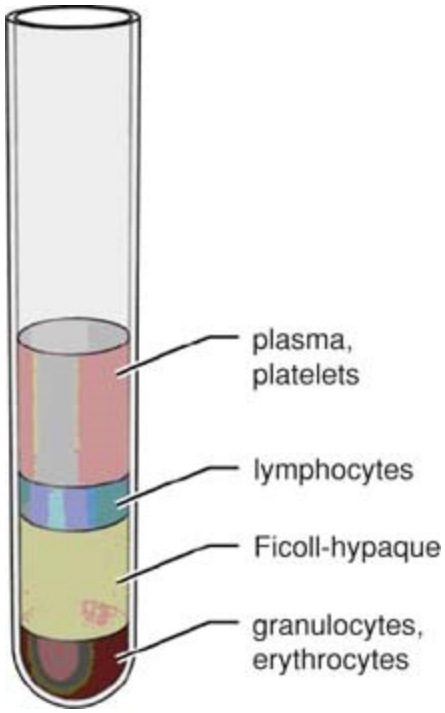
Note

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

Equipment	
Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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

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SOLUTION- 01 - Sodium Chloride (NaCl) solution

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Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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

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SOLUTION- 06 - Lysis Buffer

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

Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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

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14 Centrifuge at 400 x g, Room temperature, 00:05:00

Equipment

Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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).

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SOLUTION- 01 - Sodium Chloride (NaCl) solution

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SOLUTION- 08 - Türk solution

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17 **OPTIONAL STEP**

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

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SOLUTION- 09 - Trypan Blue solution

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Equipment

Cellometer Auto T4	NAME
Automated Cell Counter	TYPE
Nexcelom Bioscience	BRAND
Euroclone	SKU

18 **OPTIONAL STEP**

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

For this test 0,5 $\times 10^6$ PMN in 500 µL of **PBS (SOLUTION- 02)** are enough.

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SOLUTION- 02 - Phosphate Buffered Saline (PBS)

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Equipment	
BD FACS Celesta	NAME
Flow Cytometer	TYPE
Becton Dickinson	BRAND
Milan Italy BD	SKU

19 **EXPECTED RESULTS**

Expected result

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

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