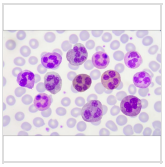

Version 2

Jun 09, 2020

Separation of Human Neutrophils (PMN) from Buffy Coat V.2

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1 *Works for me* This protocol is published without a DOI.

Mattia Di Rocco

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.

PROTOCOL CITATION

Marco Cosentino, Elisa Storelli, Alessandra Luini, Emanuela Rasini, Massimiliano LM Legnaro, Marco Ferrari, Franca Marino 2020. Separation of Human Neutrophils (PMN) from Buffy Coat. **protocols.io** https://protocols.io/view/separation-of-human-neutrophils-pmn-from-buffy-coa-bhbyj2pw

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37976

MATERIALS

NAME	CATALOG #	VENDOR
Ethylenediaminetetraacetic acid disodium salt dihydrate	ED2SS	Sigma Aldrich
Ficoll Paque PLUS	17144003-500 ml	Ge Healthcare
Fetal Bovine Serum (FBS)	ECS0180L-500 ml	EuroClone
RPMI 1640	ECM 0495L- 500 ml	EuroClone
NaCl	S9625	Sigma Aldrich

NAME	CATALOG #	VENDOR
NH4Cl	1.01145.1000	Merck Serono GmbH
KHCO3	1.04854.500	Merck Serono GmbH
Acetic Acid 100%	A6283	Sigma Aldrich
Genitian violet 1%	not available	Marco Viti

MATERIALS TEXT

Centrifuge
Cellometer (machine for automatic cell count)or Optical Microscope (for manual cell count)
Flow Cytometer

EQUIPMENT

NAME	CATALOG #	VENDOR
Cellometer Auto T4	Euroclone	
BD FACS Celesta	Milan Italy BD	

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 **Dextran solution** and mix well drawing in and out of a pipette

Dextran
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

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

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

5 ⚠

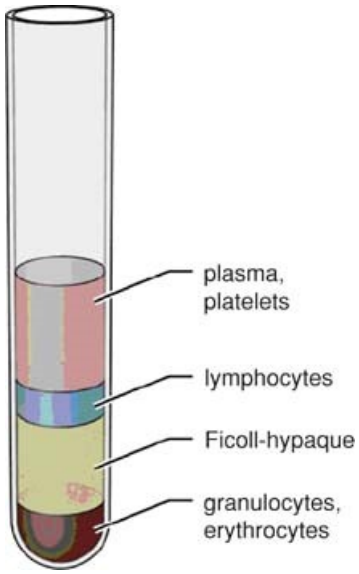
Slowly and carefully layer the supernatant from blood/dextran mixture onto the Fycoll-HyPaque media solution.

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

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

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)** and centrifuge at **400 x g, Room temperature 00:05:00**

NaCl
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

9 Aspirate the supernatant with a plastic pipette Pasteur.

10 Lyse remaining red blood cells in **5 mL** of **hypotonic Lysis Buffer** for **00:05:00** .

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**

12 Aspirate the supernatant with a plastic pipette Pasteur.

13 Resuspend the pellet in **5 mL** **NaCl** **0.15 Molarity (M)** .

NaCl
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

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

15 Aspirate the supernatant with a plastic pipette Pasteur.

16 Resuspend the cell pellet in **5 mL** **NaCl** **0.15 Molarity (M)** for cell counting.

NaCl
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

17 Mix **10 µl** of cell suspention with an equal amount of **Türk solution** (diluition factor=2) allow mixture **00:03:00** at **Room temperature** (RT).

Türk solution and Trypan Blue
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

Take **10 µl** of the mixture and place it inside a Bürker chamber and view under an optical microscope using 40x magnifiation

Count cell in each square found in the four corners and in the central square (figure 1 below), including those that lie on the bottom and left-hand perimeters, but not those that lie on the top and right hand perimeters (see figure 2 below).

Total number of cell per ml = mean number of cell x diluition factor x 10^4 (hemacitometer volume)

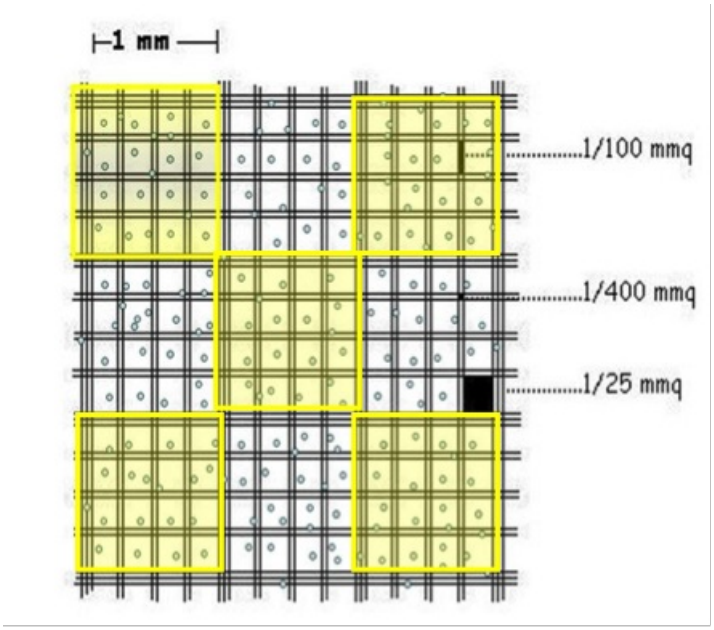


Figure 1
The gridded area of the chamber consists of nine 1 mmq squares. These squares are subdivided in three directions; 0.0625 mmq, 0.05 mmq and 0.04 mmq. The central square here in Figure 1 is further subdivided into 0.0025 mmq = 1/25 mmq squares. Count cells in 5 squares as shown.

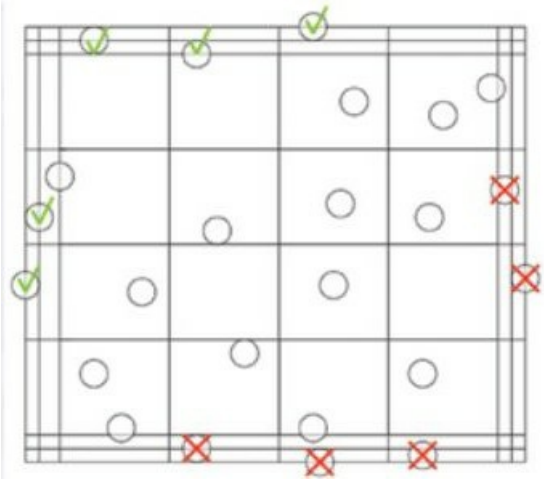


Figure 2
Concerning those cells that lay on the perimeter of the square, count following this scheme.

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OPTIONAL STEP

For automatic cell count with **Cellometer machines** use **Trypan Blue**. The machine will calculate the number of cells /ml and the % of viability.

Take **10 µl** of cell suspension and add an equal amount of Trypan Blue. Use all the volume to place it in a counting chamber. Place the chamber inside Cellometer and count.

Cellometer Auto T4
Automated Cell Counter
Nexcelom Bioscience Euroclone

Türk solution and Trypan Blue
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

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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 0,5 x10^6 PMN in **500 µl** of PBS are enough.

BD FACS Celesta
Flow Cytometer
Becton Dickinson Milan Italy BD

20 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