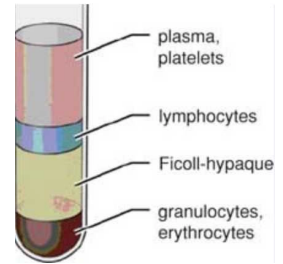


Jul 27, 2020 Version 1

PMN- 01b - Isolation of Human PMN from Whole Blood V.1

DOI

dx.doi.org/10.17504/protocols.io.bi25kgg6



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Protocol status: Working

We use this protocol and it's working

Created: July 27, 2020

Last Modified: August 03, 2020

Protocol Integer ID: 39741



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 Sci 2019 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

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)

Before start

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** and mix well by drawing in and out of a pipette.

Document






NAME

SOLUTION- 03 - Dextran solution 5%

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PREVIEW


- 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.



Note

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**.

Equipment

Allegra AVANTI 30

NAME

Centrifuge

TYPE

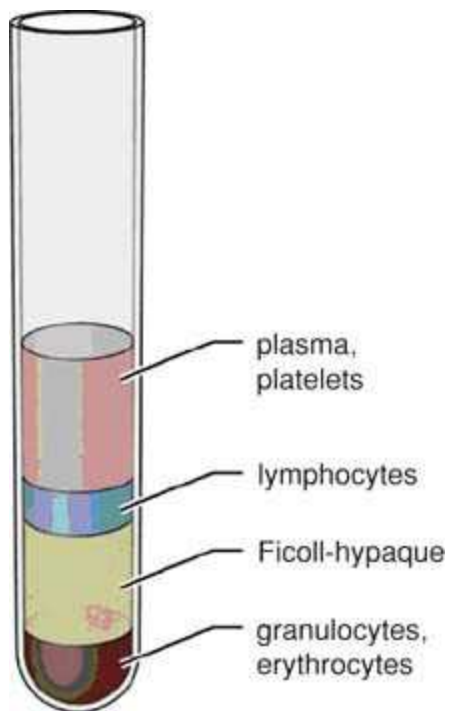
Beckman Coulter

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 μ L of **NaCl 0.15 M** and centrifuge at  400 x g, Room temperature, 00:05:00

Document



NAME

SOLUTION- 01 - Sodium Chloride (NaCl) solution

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Farmacologia Medica

PREVIEW

Equipment

Allegra AVANTI 30

NAME

Centrifuge

TYPE



Beckman Coulter

BRAND

Beckman Italy

SKU

- 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



Document




NAME

SOLUTION- 06 - Lysis Buffer

CREATED BY

Elisa Storelli

PREVIEW

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 M.**



Document




NAME

SOLUTION- 01 - Sodium Chloride (NaCl) solution

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PREVIEW

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 M** for cell counting.

Document





NAME

SOLUTION- 01 - Sodium Chloride (NaCl) solution

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PREVIEW

- 17 Mix  10 μ L of cell suspension with an equal amount of **Türk solution** (dilution factor=2) allow mixture  00:03:00 at Room temperature (RT).

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NAME

SOLUTION- 08 - Türk solution

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PREVIEW

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

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 dilution factor x 10^4
(hemacytometer volume)

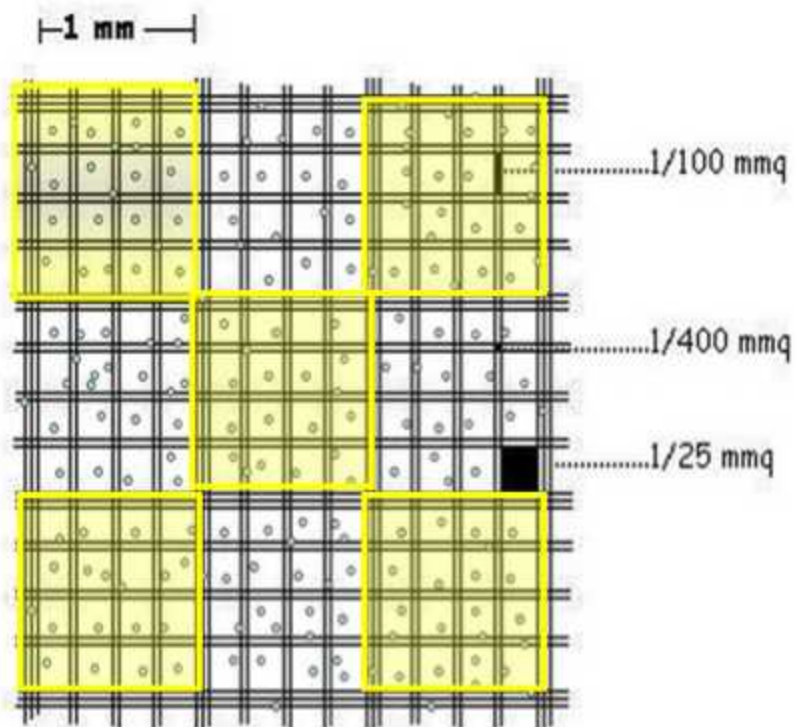


Figure 1

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.

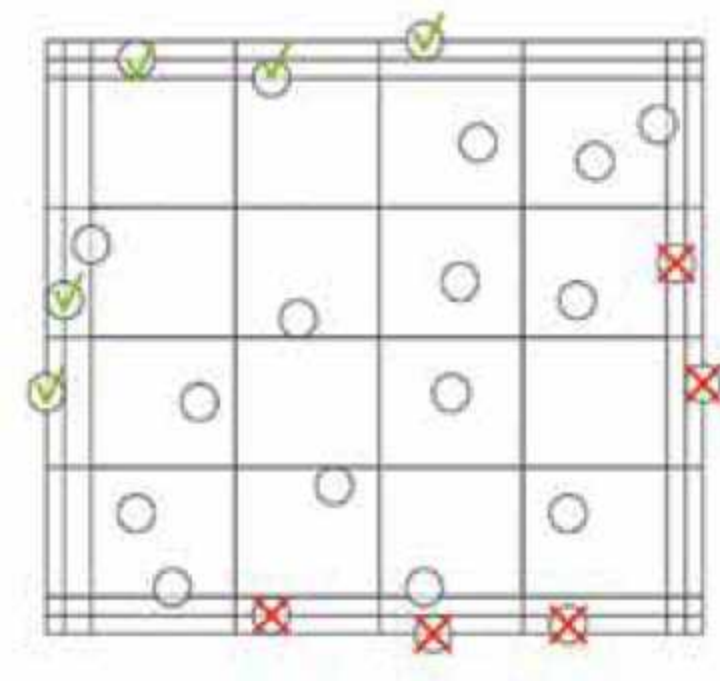



Figure 2
Concerning those cells that lay on the perimeter of the square, count following this scheme.

18 **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.



Document



NAME

SOLUTION- 09 - Trypan Blue solution

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PREVIEW

Equipment

Cellometer Auto T4

NAME

Automated cell counter

TYPE

Nexcelom Bioscience

BRAND

EuroClone

SKU

19 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** are enough.



Document



NAME

SOLUTION- 02 - Phosphate Buffered Saline (PBS)

CREATED BY

Elisa Storelli

PREVIEW

Equipment

BD FACSCelesta

NAME

Flow Cytometer

TYPE

Becton Dickinson, Milan Italy

BRAND

BD

SKU

20 **EXPECTED RESULTS**

Expected result

VIABILITY: the expeted viability by Trypan Blue shuold be $\geq 90\%$ **CELL YIELD:** $\pm 1 \times 10^6$ cells starting from 1 mL of Whole Blood