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Version 2

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

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Protocol status: Working

We use this protocol and it's working

Created: November 21, 2020

Last Modified: November 21, 2020

Protocol Integer ID: 44760

Keywords: constituent cannabidiol inhibit human polymorphonuclear leukocyte function, isolation of human pmn, human polymorphonuclear leukocyte, production by human polymorphonuclear leukocyte, granulocytes from human blood, mononuclear cell, human pmn, granulocyte

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9625**

 KHCO3 **Merck Catalog #1.04854.500**

 EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #ED2SS**

 Acetic Acid 100% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A6283**

 Gentian violet 1% **Marco Viti Catalog #not available**

 NH4Cl **Merck 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 (**SOLUTION- 03**) and mix well by 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  00:30:00 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

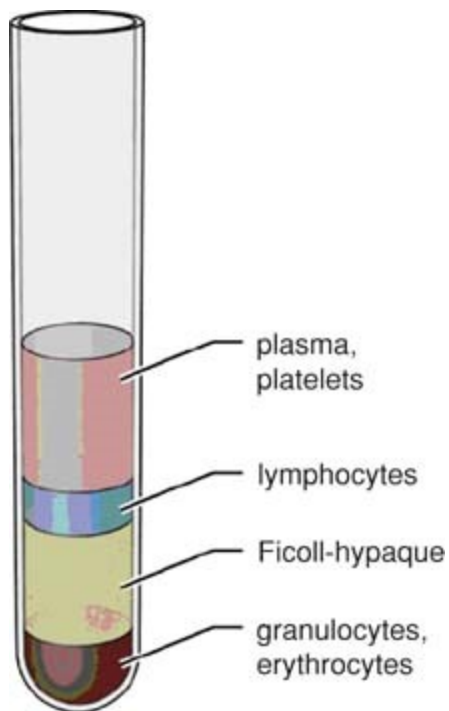
Beckman Coulter

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 (**SOLUTION- 01**) and centrifuge at  400 x g, Room temperature, 00:05:00

Document

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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 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

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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 (**SOLUTION- 01**).



Document

NAME

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 M (**SOLUOTIN- 01**) for manual cell counting using Türk solution (SOLUTION- 08). Follow protocol **CELL COUNT- 02**.

**Document**

NAME

SOLUTION- 01 - Sodium Chloride (NaCl) solution

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NAME

SOLUTION- 08 - Türk solution

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[Preview](#)**17 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.



Document

NAME

SOLUTION- 02 - Phosphate Buffered Saline (PBS)

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Equipment

BD FACSCelesta

NAME

Flow Cytometer

TYPE

Becton Dickinson, Milan Italy

BRAND

BD

SKU

18 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