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

Created: Nov 21, 2020

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

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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 INSTRUCTIONS

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.

Document

NAME

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 $5\ \mu\text{L}$ of NaCl 0.15 M (**SOLUTION- 01**) and centrifuge at $400 \times g$, Room temperature, 00:05:00

Document

NAME

SOLUTION- 01 - Sodium Chloride (NaCl) solution

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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 (**SOLUTION- 06**) for 00:05:00

Document	
	NAME
	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**.

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NAME
SOLUTION- 01 - Sodium Chloride (NaCl) solution

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Document

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 expected viability by Trypan Blue should be $\geq 90\%$

CELL YIELD: $\pm 1 \times 10^6$ cells starting from 1 mL of Whole Blood