Version 4

Nov 21, 2020

PBMC- 01b Isolation of human PBMC from Whole Blood V.4

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1 Works for me dx.doi.org/10.17504/protocols.io.bpxmmpk6

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ABSTRACT

Separation and purification of PBMC from FRESH BLOOD: list of published work using this protocol

Kustrimovic, N., Comi, C., Magistrelli, L., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Minafra, B., Riboldazzi, G., Sturchio, A., Mauri, M., Bono, G., Marino, F., & Cosentino, M. (2018). Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. Journal of neuroinflammation, 15(1), 205. https://doi.org/10.1186/s12974-018-1248-8

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Cosentino M., Ferrari M., Kustrimovic N., Rasini E., Marino F. (2015). Influence of dopamine receptor gene polymorphisms on circulating T lymphocytes: A pilot study in healthy subjects. Human immunology, 76, 10, 747-752. https://doi.org/10.1016/j.humimm.2015.09.032

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PROTOCOL CITATION

Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. PBMC- 01b Isolation of human PBMC from Whole Blood. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpxmmpk6
Version created by Farmacologia Medica

KEYWORDS

PBMC, Fresh Blood, Neuroimmune-Pharmacology, Parkinson's Disease, Cell isolation, Primary cell culture

CREATED

Nov 21, 2020

Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino (11/21/2020). PBMC- 01b Isolation of human PBMC from Whole Blood. https://dx.doi.org/10.17504/protocols.io.bpxmmpk6

LAST MODIFIED

Nov 21, 2020

OWNERSHIP HISTORY

Nov 21, 2020 Elisa Storelli Center for Research in Medical Pharmacology, University of Insubria

PROTOCOL INTEGER ID

44749

MATERIALS TEXT

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**

Trypan Blue solution 0.4% **Sigma Aldrich Catalog #T8154- 100 ml**

NaCl **Sigma Aldrich Catalog #S9625**

Na2HPO4*7H2O **Merck Serono GmbH Catalog #1.06574.1000**

NaH2PO4 **Merck Serono GmbH Catalog #1.06346.0500**

NH4Cl **Merck Serono GmbH Catalog #1.01145.1000**

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**

Instrumentation required:

- Laminar flow hood
- Autoclave

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have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. Journal of neuroinflammation, 15(1), 205. https://doi.org/10.1186/s12974-018-1248-8

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BEFORE STARTING

If you need to obtain **PBMC for cell culture**, make sure you are using **sterile PBS, culture medium, filtered Lysis Buffer and sterile plastic disposables as well.** Moreover, work under laminar flow hood when you are processing samples. Otherwise, use non-sterile solutions and plastic disposables, and process samples in cell isolation laboratory.

ALL REAGENTS USED IN THIS PROTOCOL MUST BE AT ROOM TEMPERATURE!

1 Put the needed amount of blood sampl into a **50 mL** conical tube.

2 Add an equal volume of **PBS 1X** and mix well.

SOLUTION- 02 - Phosphate Buffered Saline (PBS)
by Farmacologia Medica

3 Place **3 mL** of FICOLL in a **15 mL** conical tube.

4 ⚠

Carefully layer **12 mL** of diluted blood on FICOLL with a glass Pasteur Pipette to a final volume of 15 ml as shown in the figure below.

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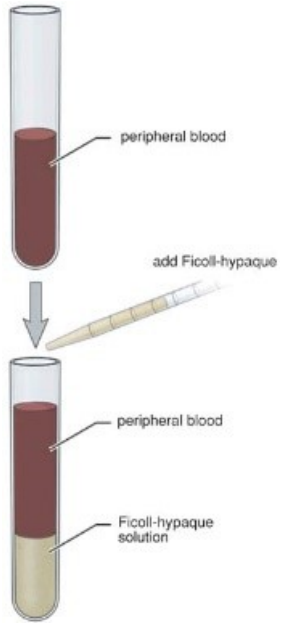
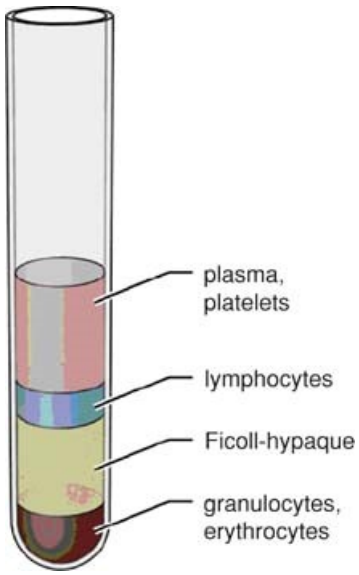


5 Centrifuge samples **400 x g, 00:40:00** at room temperature (RT) without break.

Allegra AVANTI 30
Centrifuge
Beckman Coulter **Beckman Italy**

6 After centrifugation, take out the tubes carefully to not disturb the mononuclear cell layer that appears as a white, cloudy band between the plasma and FICOLL as shown in the figure below.



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7 ⚠

Carefully with a glass Pasteur pipette transfer the mononuclear lymphocyte cell layer to another 15 ml conical tube.

8 Wash the isolated PBMC with **PBS/FBS 2%** to a final volume of **10 mL** and centrifuge at **600 x g, 00:10:00** at RT.

SOLUTION- 05 - Wash solution (PBS/FBS) for PBMC
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

9 Remove supernatants, resuspend pellet in **1 mL** of **Lysis Buffer** and add another **9 mL** of **Lysis Buffer**. Immediately centrifuge tubes at **300 x g, 00:10:00** at RT.

SOLUTION- 06 - Lysis Buffer
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

10 Remove supernatant and resuspend pellet in **10 mL** of **PBS/FBS 2%** and centrifuge at **600 x g, 00:10:00** at RT.

SOLUTION- 05 - Wash solution (PBS/FBS) for PBMC
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11 Remove supernatant and resuspend the obtained pellet in **10 mL** of **RPMI/FBS 10%** for cell counting.

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SOLUTION- 04 - Wash solution (RPMI/FBS) for PBMC
by Farmacologia Medica

12 **For manual cell count use Türk solution for checking purity.**

Follow protocol **CELL COUNT- 02.**

SOLUTION- 08 - Türk solution
by Farmacologia Medica

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OPTIONAL STEP

For automatic cell count with Cellometer machine use Trypan Blue.

Follow protocol **CELL COUNT- 03**.

SOLUTION- 09 - Trypan Blue solution
by Farmacologia Medica

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If needed, check the purity of PBMC suspension by using morphological parameter of the flow cytometer.

For this test $0.5x10^6$ PBMC in 500 µl of PBS are enough.

BD FACS Celesta
Flow Cytometer
Becton Dickinson **Milan Italy BD**

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15 Expected results

VIABILITY - The expected viability by Trypan Blue should be ≥90 %.

PURITY - The PBMC suspension obtained should contain at least 80% of lymphocytes, 10-15% of monocytes and few contaminant PMN cells (≤ 5%) as confirmed by flow cytometry.

YIELD - The expected amount of PBMCs should be ± 28,5x10^6 starting from 25 ml of fresh blood.

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