Version 2

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PBMC- 01a - Isolation of Human PBMC from Buffy Coat V.2

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

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ABSTRACT

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

- Kustrimovic, N., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Comi, C., Mauri, M., Minafra, B., Riboldazzi, G., Sanchez-Guajardo, V., Marino, F., & Cosentino, M. (2016). Dopaminergic Receptors on CD4+ T Naive and Memory Lymphocytes Correlate with Motor Impairment in Patients with Parkinson's Disease. Scientific reports, 6, 33738. https://doi.org/10.1038/srep33738

- 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

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KEYWORDS

PBMC, Buffy Coat, Neuroimmune-Pharmacology, Parkinson's Disease, Cell isolation, Primary cell culture

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MATERIALS TEXT

MATERIALS

Fetal bovine serum

(FBS) **BioWest Catalog #S181B-500**

Ficoll Paque PLUS **Ge**

Healthcare Catalog #17144003-500 ml

RPMI

1640 **EuroClone Catalog #ECM 0495L- 500 ml**

Trypan Blue Solution 0.4% **Thermo Fisher**

Scientific Catalog #15250061

Instrumentation required:

- Laminar flow hood
- Optical Microscope (manual cell count)

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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 sample from buffy coat into a 50 ml conical tube.

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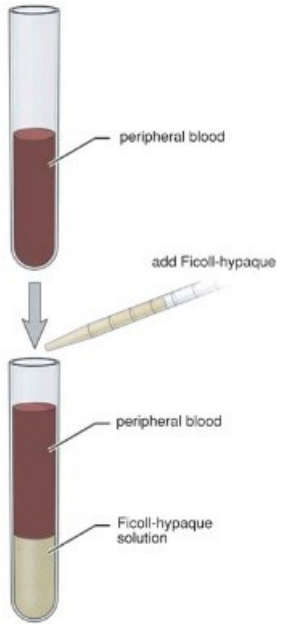
2 Add an equal volume of **PBS 1X** and mix well.

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 the FICOLL with a glass Pasteur Pipette to a final volume of 15 ml as shown in the figure below.



5 Centrifuge samples **400 x g, 00:40:00** **without break.**

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

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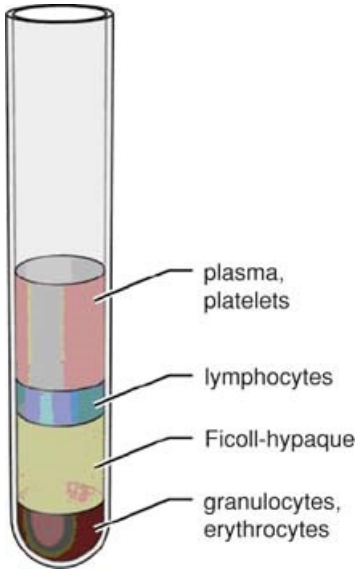
6 After centrifugation, take out the tubes carefully to not disturb the mononuclear cell layer that appears as a white, cloudy band between the plasma and FICOLL as shown in the figure below.



7 ⚠

Carefully with a glass Pasteur pipette transfer 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 **300 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

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

9 Remove supernatants, resuspend pellet in **1 mL** of **Lysis Buffer** and add another **9 mL** of **Lysis Buffer.**

Immediately centrifuge the tubes at **100 x g, 00:10:00** at RT.

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

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

10 Remove supernatant and resuspend pellet in **10 mL** **PBS/FBS 2%** and centrifuge at **300 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

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

11 Remove supernatant and resuspend the obtained pellet in **10 mL** of **RPMI/FBS 10%** for cell counting.

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**

Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino (11/21/2020). PBMC- 01a - Isolation of Human PBMC from Buffy Coat. https://dx.doi.org/10.17504/protocols.io.bpxjmpkn

SOLUTION- 08 - Türk solution
by Farmacologia Medica

13

OPTIONAL STEP

For automatic cell count with Cellometer machine use Trypan Blue.

Follow protocol **CELL COUNT- 03**.

Cellometer Auto T4
Automated cell counter
Nexcelom Bioscience **EuroClone**

SOLUTION- 09 - Trypan Blue solution
by Farmacologia Medica

14

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**

15 Expected results

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

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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 ± $100x10^6$ starting from 25 ml of buffy coat.

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