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PBMC- 01a - Isolation of Human PBMC from Buffy Coat

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1 Works for me dx.doi.org/10.17504/protocols.io.biw2kfge

Farmacologia Medica

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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KEYWORDS

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

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MATERIALS

NAME	CATALOG #	VENDOR
Fetal bovine serum (FBS)	S181B-500	BioWest
Ficoll Paque PLUS	17144003-500 ml	Ge Healthcare
RPMI 1640	ECM 0495L- 500 ml	EuroClone
Trypan Blue Solution 0.4%	15250061	Thermo Fisher Scientific

MATERIALS TEXT

Instrumentation required:

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

EQUIPMENT

NAME	CATALOG #	VENDOR
Cellometer Auto T4	EuroClone	
BD FACS Celesta	Milan Italy BD	
Allegra AVANTI 30	Beckman Italy	Beckman Coulter

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.

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

SOLUTION- 02 - Phosphate Buffered Saline (PBS)
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

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

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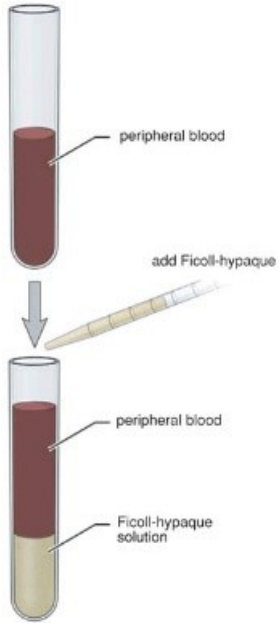
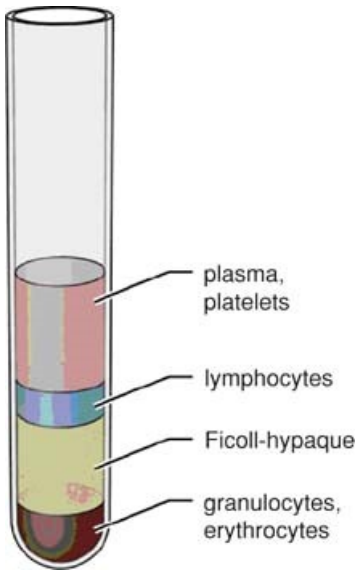
as shown in the figure below.



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

Allegra AVANTI 30
Centrifuge
Beckman Coulter Beckman Italy

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 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.

SOLUTION- 06 - Lysis Buffer
by Farmacologia Medica

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.

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

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

Mix 10 µl of cell suspention with an equal amount of Türk solution (dilution factor = 2), allow mixture 3 min at room temperature.
Take 10 µl of the mixture and place it inside a Bürker chamber and view under an optical microscope using 40X magnification.

Count the cells in each square found in the four corners and in the central square (see 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 cells per ml = mean number of cells x dilution factor x 104 (hemacytometer volume).

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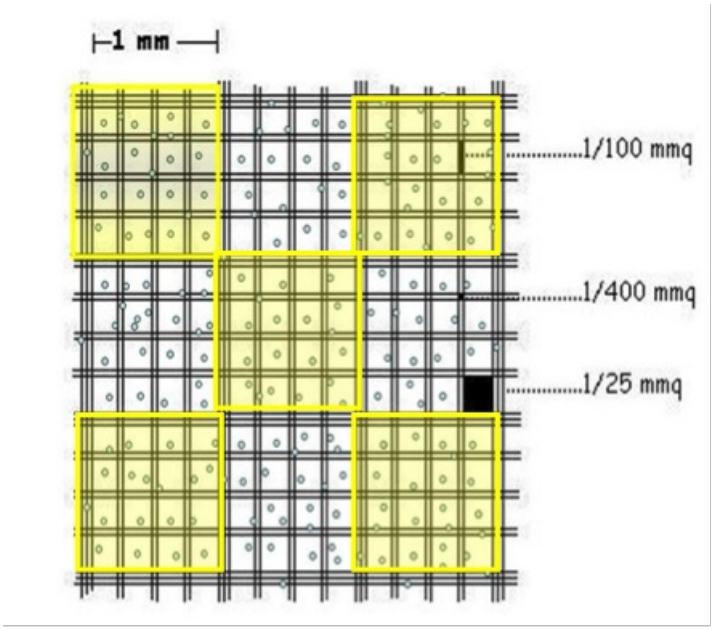

Figure 1
The gridded area of the chamber consists of nine 1 mmq squares. These squares are subdivided in three directions; 0.0625 mmq, 0.05 mmq and 0.04 mmq. The central square here in Figure 1 is further subdivided into 0.0025 mmq = 1/25 mmq squares. Count cells in 5 squares as shown.

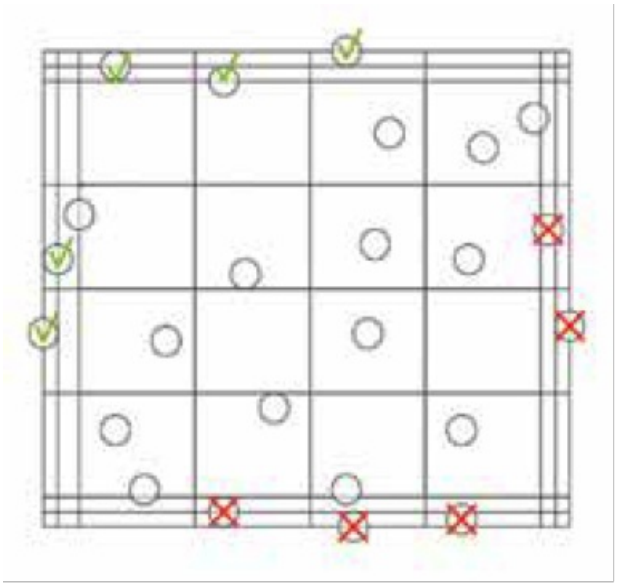

Figure 2
Concerning those cells that lay on the perimeter of the square, count following this scheme.

SOLUTION- 08 - Türk solution
by Farmacologia Medica

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OPTIONAL STEP

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For automatic cell count with Cellometer machine use Trypan Blue.

The machine will calculate the n°of cells/ml and the % of viability.
Take **10 µl** of cell suspention 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.

Cellometer Auto T4
Automated cell counter
Nexcelom Bioscience EuroClone

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

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

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