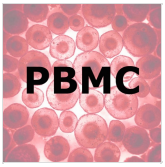


May 05, 2020

# Separation and purification of human PBMC from FRESH BLOOD

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1 Works for me dx.doi.org/10.17504/protocols.io.bfxmjpk6

Elisa Storelli
Center for Research in Medical Pharmacology, University of I...

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

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

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Ficoll Paque PLUS | 17144003-500 ml | Ge Healthcare |
| Fetal Bovine Serum (FBS) | ECS0180L-500 ml | EuroClone |
| RPMI 1640 | ECM 0495L- 500 ml | EuroClone |
| Trypan Blue solution 0.4% | T8154- 100 ml | Sigma Aldrich |

MATERIALS TEXT

**Instrumentation required:**

- Laminar flow hood
- Centrifuge
- Cellometer (automated cell counter) or Optical Microscope (manual cell count)
- Flow Cytometer
- Autoclave

EQUIPMENT

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



**Citation:** Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino (05/05/2020). Separation and purification of human PBMC from FRESH BLOOD. https://dx.doi.org/10.17504/protocols.io.bfxmjpk6

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!**

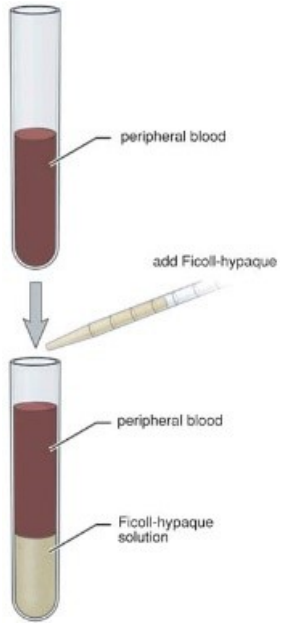
1 Put the needed amount of blood sampl into a **50 ml** conical tube.

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

PBS 1X
**by Marco Ferrari,**
**University of Insubria**

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.




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



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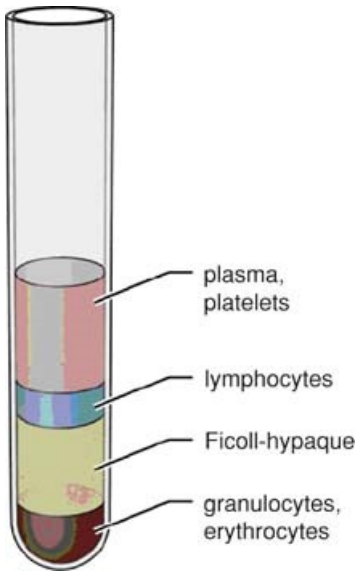
Allegra AVANTI 30
Centrifuge
**Beckman Coulter Beckman Italy**

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.




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.

RPMI - FBS and PBS - FBS
**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.



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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.

RPMI - FBS and PBS - FBS
by Elisa Storelli,
Center for Research in Medical Pharmacology, University of Insubria

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

RPMI - FBS and PBS - FBS
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 $10^4$ (hemacytometer volume).**



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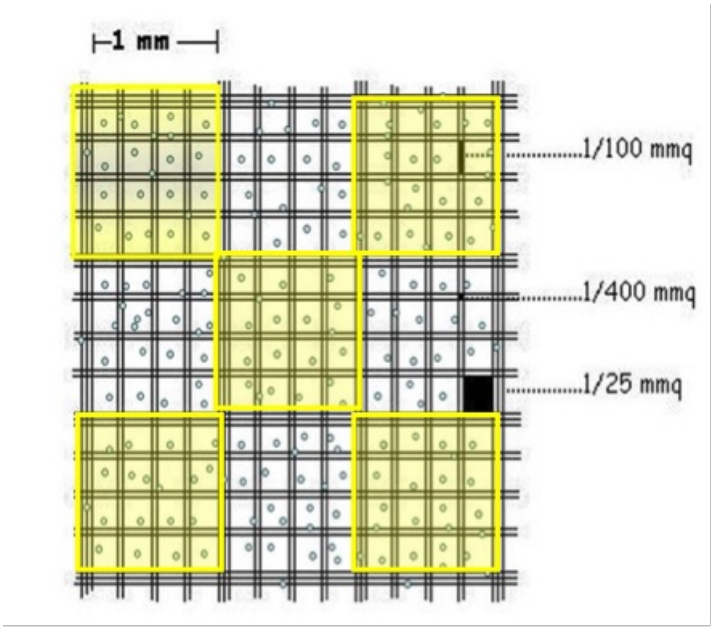


Figure 1

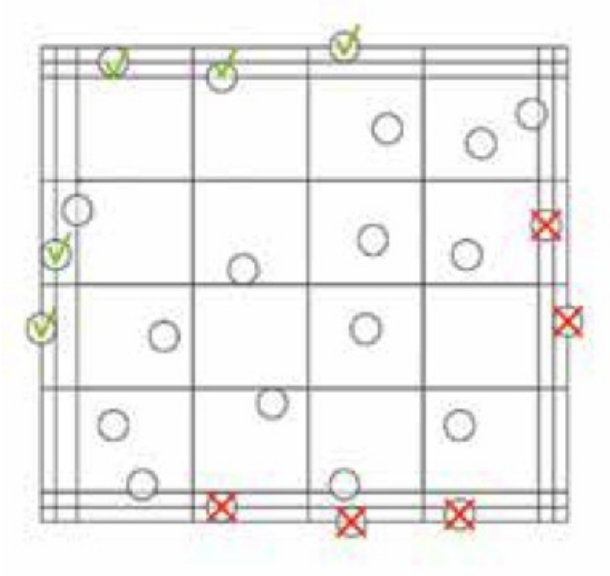

Figure 2

Trypan blue and Turk solution
**by Marco Ferrari,**
**University of Insubria**

## 13 OPTIONAL STEP

**For automatic cell count with Cellometer machine use Trypan Blue.** The machine will calculate the n° of cells/ml and the % of viability.



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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**

Trypan blue and Turk solution
**by Marco Ferrari,**
**University of Insubria**

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 %.

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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