VERSION 1

MAY 05, 2020

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bfxmjpk6

Protocol Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino 2020. Separation and purification of human PBMC from FRESH BLOOD.

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<https://dx.doi.org/10.17504/protocols.io.bfxmjpk6>

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

Created: May 04, 2020

Last Modified: May 05, 2020

🌐 Separation and purification of human PBMC from FRESH BLOOD V.1

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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>

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>

PROTOCOL integer ID:
36557

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

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

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

Instrumentation required:

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

BEFORE START INSTRUCTIONS

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.

Document

NAME
PBS 1X

CREATED BY
Marco Ferrari

PREVIEW

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.

Equipment	
Allegra AVANTI 30	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

- 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.

Document

NAME

RPMI - FBS and PBS - FBS

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- 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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NAME

Lysis Buffer

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

Document	
	NAME RPMI - FBS and PBS - FBS
CREATED BY Elisa Storelli	PREVIEW

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

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	NAME RPMI - FBS and PBS - FBS
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- 12 **For manual cell count use Türk solution for checking purity.**

Mix 10 µL of cell suspension 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).

Figure 1

Figure 2

Document

NAME

Trypan blue and Turk solution

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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.

Take 10 μ L of cell suspension 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.

Equipment	
Cellometer Auto T4	NAME
Automated Cell Counter	TYPE
Nexcelom Bioscience	BRAND
Euroclone	SKU

Document	
	NAME
Trypan blue and Turk solution	
CREATED BY	
Marco Ferrari	
	PREVIEW

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

For this test 0.5×10^6 PBMC in 500 μ l of PBS are enough.

Equipment	
BD FACS Celesta	NAME
Flow Cytometer	TYPE
Becton Dickinson	BRAND
Milan Italy BD	SKU

15 Expected results

Expected result

VIABILITY - The expected viability by Trypan Blue should be $\geq 90\%$.

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

YIELD - The expected amount of PBMCs should be $\pm 28,5 \times 10^6$ starting from 25 ml of fresh blood.