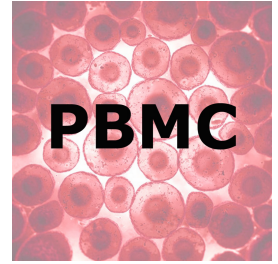


Nov 21, 2020 Version 2

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

DOI

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** November 21, 2020

**Last Modified:** November 21, 2020

**Protocol Integer ID:** 44747

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

## 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>

## Materials

### 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)

## Before start

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.

### Document





NAME

**SOLUTION- 02 - Phosphate Buffered Saline (PBS)**

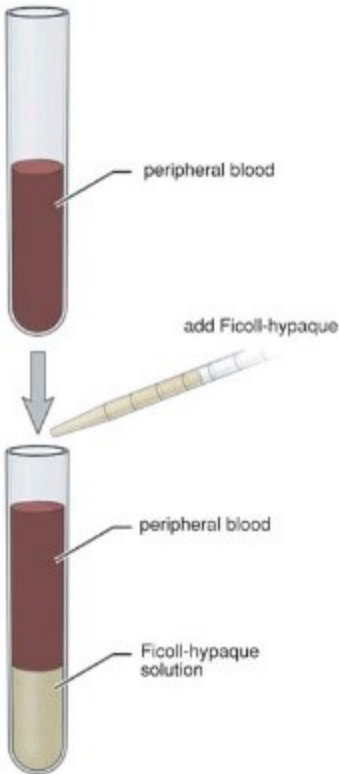
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**PREVIEW**

- 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  **without break.**

#### Equipment

**Allegra AVANTI 30**

NAME

Centrifuge

TYPE

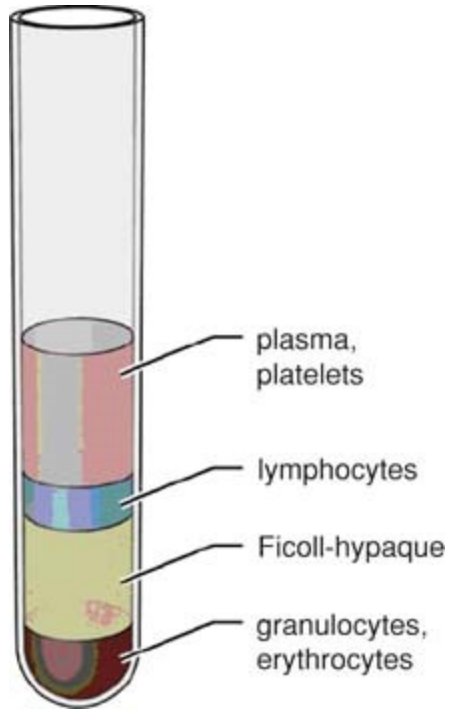
Beckman Coulter



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 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.



### Document



NAME

**SOLUTION- 05 - Wash solution (PBS/FBS) for PBMC**




CREATED BY

**Elisa Storelli**


**PREVIEW**

### Equipment

<b>Allegra AVANTI 30</b>	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU



- 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.

### Document


	NAME
	<b>SOLUTION- 06 - Lysis Buffer</b>
CREATED BY	<b>PREVIEW</b>
<b>Elisa Storelli</b>	

### Equipment

<b>Allegra AVANTI 30</b>	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU


10 Remove supernatant and resuspend pellet in  10 mL **PBS/FBS 2%** and centrifuge at  300 x g, 00:10:00 at RT.

### Document

	NAME
	<b>SOLUTION- 05 - Wash solution (PBS/FBS) for PBMC</b>
CREATED BY	<b>PREVIEW</b>
<b>Elisa Storelli</b>	

Equipment

<b>Allegra AVANTI 30</b>	NAME
Centrifuge	TYPE
Beckman Coulter	BRAND
Beckman Italy	SKU

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

Document



NAME

**SOLUTION- 04 - Wash solution (RPMI/FBS) for PBMC**

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**PREVIEW**

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

Follow protocol **CELL COUNT- 02**



Document



NAME

**SOLUTION- 08 - Türk solution**

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PREVIEW

13 **OPTIONAL STEP**



**For automatic cell count with Cellometer machine use Trypan Blue.**

Follow protocol **CELL COUNT- 03**.

Equipment

**Cellometer Auto T4**

NAME

Automated cell counter

TYPE

Nexcelom Bioscience

BRAND

EuroClone

SKU

Document



NAME

**SOLUTION- 09 - Trypan Blue solution**

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**PREVIEW**

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

For this test  $0.5 \times 10^6$  PBMC in 500  $\mu$ 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  $\pm 100 \times 10^6$  starting from 25 ml of buffy coat.