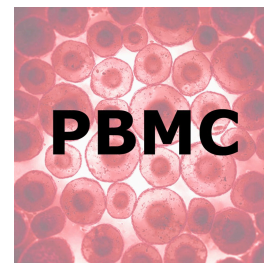


Nov 21, 2020

Version 4

PBMC- 01b Isolation of human PBMC from Whole Blood V.4



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: 44749

Keywords: PBMC, Fresh Blood, Neuroimmune-Pharmacology, Parkinson's Disease, Cell isolation, Primary cell culture, isolation of human pbmc, human pbmc, patients with parkinson, purification of pbmc, parkinson, pbmc, influence of dopamine receptor gene polymorphism, dopaminergic receptor, dopamine receptor gene polymorphism, dopaminergic receptors on cd4, journal of neuroinflammation, whole blood separation, treg in drug,

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

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% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8154- 100 ml**
- ✕ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9625**
- ✕ Na₂HPO₄*7H₂O **Merck Catalog #1.06574.1000**
- ✕ NaH₂PO₄ **Merck Catalog #1.06346.0500**
- ✕ NH₄Cl **Merck Catalog #1.01145.1000**
- ✕ KHCO₃ **Merck Catalog #1.04854.500**
- ✕ EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #ED2SS**
- ✕ Acetic Acid 100% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A6283**
- ✕ Gentian violet 1% **Marco Viti Catalog #not available**

Instrumentation required:

- Laminar flow hood
- Autoclave


Troubleshooting

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 sampl 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)

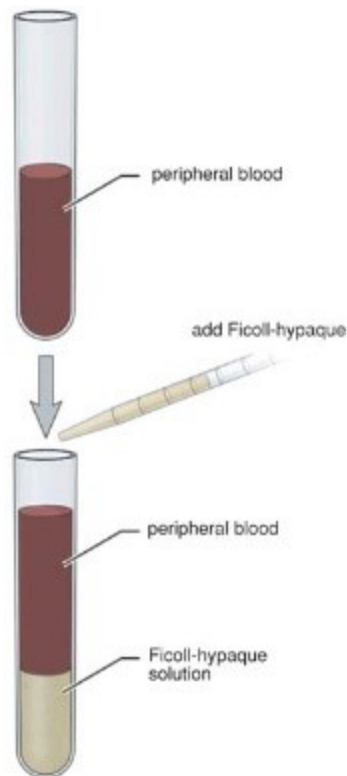
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Preview

- 3 Place  3 mL of FICOLL in a  15 mL conical tube.
- 4 Carefully layer  12 mL of diluted blood on FICOLL with a glass Pasteur Pipette to a final volume of 15 ml as shown in the figure below.





- 5 Centrifuge samples at room temperature (RT) without break.

Equipment

Allegra AVANTI 30

NAME

Centrifuge

TYPE

Beckman Coulter

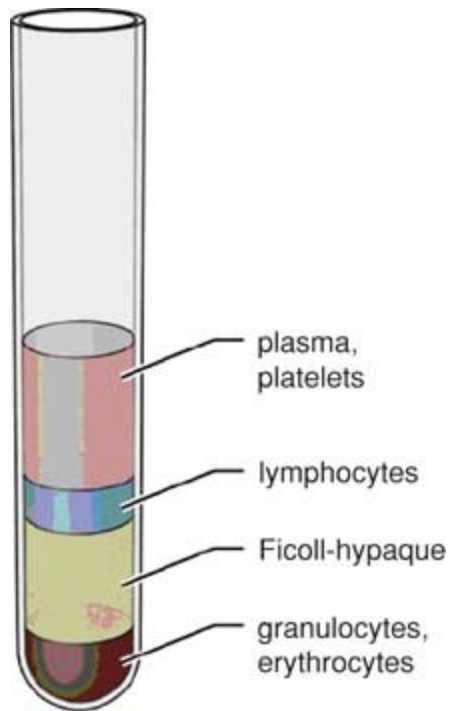
BRAND




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

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

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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.

Document



NAME

SOLUTION- 06 - 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

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

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- 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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- 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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13 OPTIONAL STEP**For automatic cell count with Cellometer machine use Trypan Blue.**Follow protocol **CELL COUNT- 03**.**Document**

NAME

SOLUTION- 09 - Trypan Blue solution

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- 14** 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.