



Montreal Neurological Institute and Hospital
McGill University

CBIG-02-002

**PBMC ISOLATION FROM WHOLE BLOOD
(LEUCOSEP METHOD)**

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	30-Apr-2020

2. SCOPE AND APPLICATION

This protocol is to be used for the *in vitro* isolation of human peripheral blood mononuclear cells (PBMC) using the Leucosep method.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-002 PBMC Isolation from Whole Blood (Leucosep Method)*.

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-02-004 DNA Extraction from Whole Blood (Accelerated Method)
2. C-BIG Repository: CBIG-02-009 Preparation and Testing of Human AB Serum for Subsequent Storage and Use
3. C-BIG Repository: CBIG-03-001 Different Methods to Count Cells
4. C-BIG Repository: CBIG-03-002 RPM Conversion

3.2 Reference to External SOPs or Documents

1. Greiner Bio-One, Instruction Manual for Leucosep, Revision 01 February 2019 - F071043

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who process human peripheral blood samples for PBMC isolation using the Leucosep method. All personnel who read this SOP should sign the form found in the reading log binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
C-BIG	Clinical Biological Imaging and Genetic Repository
DMSO	Dimethyl Sulfoxide
GTT	Green Top Tubes (vacutainer)
HI HuAB serum	Heat Inactivated Human AB Serum
Min	Minutes
mL	Milliliters
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PTT	Purple/Lavender Top Tube (vacutainer)
QA	Quality Assurance
QC	Quality Control
RPM	Rotation Per Minute
RT	Room Temperature

SOP	Standard Operating Procedure
µL	Microliters
°C	Degree Celsius

6. MATERIALS AND EQUIPMENT

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

NOTE: All disposable items are sterile (gamma-irradiated) unless otherwise specified. All equipment, disposables or reagents can be substituted with equivalent materials following evaluations and approval, unless specified otherwise.

NOTE: All sample contact materials must be suitable for RNA work (i.e. RNase-free). Use clean gloves at all times to prevent inadvertent RNase contamination during processing. All equipment, disposables or reagents can be substituted with equivalent materials following evaluation and approval, unless specified otherwise.

Material/Equipment	Material/Equipment (site specific)
Blood collection tube with K2 EDTA, 10 mL, purple/lavender top	BD Diagnostic; Cat # 366643
Blood collection tube with Sodium Heparin, 10 mL, green top	BD Diagnostic; Cat # 366480
Cryovials round-bottom, 1.8 mL	Nunc Biobanking and Cell Culture Cryogenic Tubes, Thermo Scientific; Cat # 368632

Disposable serological pipets 5 mL	
Disposable serological pipets, 10 mL	
Disposable transfer pipets	
Pipet tips, 200 µL	
Pipet tips, 1000 µL	
Leucosep tube, 50 mL	Grenier Bio-One, Leucosep centrifuge tubes, 50 mL; Cat # 227290
Flip cap polypropylene conical tubes, 50 mL	Thermo Scientific, Nunc EZ Flip Cap, 50 mL; Cat # 362696
Pipet-aid	
Class II biological safety cabinet	
Micropipets, range 100 to 1000 µL	
Ficoll-Paque Plus	GE Healthcare; Cat # 17-1440-03
Phosphate buffered saline (1X), pH 7.4, CA ²⁺ and Mg ²⁺ free	Wisent; Cat # 311-010-CL
DMSO	Fisher Scientific; Cat # D1391
Centrifuge with swinging bucket	Eppendorf centrifuge 5810, Cat # 022625004; with rotor S-4-104, Cat # 5820759003
Human Serum	Human Serum from human male AB plasma, Sigma; Cat # H4522
Nalgene Cryo 1°C “Mr. Frosty” Freezing Container	Nalgene Nunc; Cat # 5100-0001
2-propanol	
-80°C Freezer	
Liquid Nitrogen Tank	

7. PROCEDURES

PRECAUTIONS: All biological samples derived from human source are considered to be biohazardous. Use appropriate precautions when working with such samples (i.e. personal protection equipment such as gloves, lab coat and safety glasses). All waste (samples and related contact materials) must be placed in marked biohazardous waste containers and disposed of under hospital guidelines.

NOTE: Best results are obtained when whole blood is processed promptly. When possible, processing should be done on the same day as the blood draw. If the blood is not processed immediately after the blood draw, put the vacutainers tubes on the shaker until ready to process. If it is not possible to process the same day, put the vacutainers on the shaker for 30 min, then place them in the credo box RT overnight. The next morning, prior to sample processing, shake the green and purple vacutainers for 30 min.

NOTE: Vacutainers containing less than 4 mL of blood should not be used for PBMC isolation. They need to be discarded or alternatively if the blood is collected in PTT vacutainers, it may be possible to extract DNA from it. Refer to SOP *CBIG-02-004 DNA Extraction from Whole Blood (Accelerated Method)* for more information.

7.1 Leucosep Method

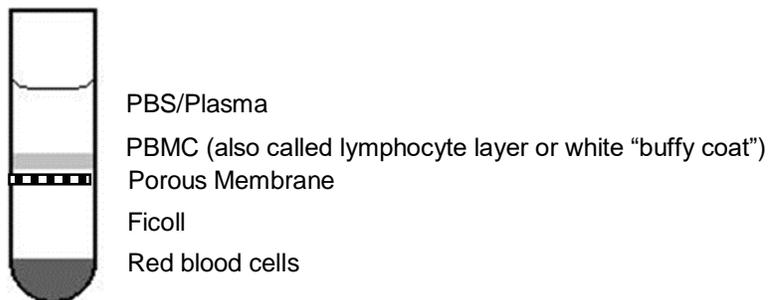
1. Fill the Leucosep tube with 15 mL of Ficoll.
2. Centrifuge the Leucosep tube at RT for 30 seconds at 1000Xg using a centrifuge with swinging bucket. The Ficoll will then be below the porous membrane.

3. Distribute whole blood from any number of GTT or PTT equally into the Leucosep tube so that each tube contains between 10-20 mL of blood (approximately two full 10 mL GTTs or PTTs per Leucosep tube).

NOTE: Dilution of the sample material with PBS is not necessary, but highly recommended; it can help to improve the results of the separation. For blood a dilution ratio of 1:2 is recommended.

4. Rinse blood collection tubes with PBS and distribute into Leucosep tube.
5. Complete each Leucosep tube with PBS up to 50 mL.
6. Centrifuge at RT for 10 min at 1000Xg with medium acceleration and no brake.

NOTE: After centrifugation, the following layers are observed:



7. Pour the layer above the porous membrane of the Leucosep tube or transfer the PBMC layer with a transfer pipette into a new 50 mL flip cap polypropylene conical tube.
8. Complete the volume with PBS up to 50 mL and centrifuge at RT for 10 min at 250Xg.

9. Remove the supernatant and loosen the cell pellet by tapping the tube with your finger or “raking” gently against an undulated surface.
10. Pool cell pellets together in a final volume of 10 mL of PBS. If needed to count cells, please refer to SOP *CBIG-03-001 Different Methods to Count Cells*. If not proceed to step 11.
11. Complete the volume with PBS up to 50 mL and centrifuge at RT for 10 min at 250Xg.

NOTE: At this point, PBMC suspension can be either frozen down for cryopreservation see section 7.3 for further details or used them fresh for your purposes.

7.2 Summary Table

Steps
1. Fill Leucosep tube with 15 mL of Ficoll
2. Centrifuge for 30 seconds at 1000Xg
3. Evenly distribute whole blood into 50 mL Leucosep tube and add an equal volume of PBS
4. Centrifuge for 10 min at 1000Xg
5. Collect PBMC layer and transfer to a new 50 mL flip cap polypropylene conical tube
6. Complete to 50 mL total volume with PBS
7. Centrifuge for 10 min at 250Xg and discard supernatant
8. Loosen cell pellets and pool them in a final volume of 10 mL PBS (count cells if needed)

9. Complete to 50 mL total volume with PBS
10. Centrifuge for 10 min at 250Xg
11. Freeze PBMC or use fresh

7.3 Freezing PBMC

1. Discard supernatant and loosen cell pellet by tapping the tube with your finger or “raking” gently against an undulated surface.
2. Prepare Solution A (HI HuAB serum) and Solution B (HI HuAB serum + DMSO) – see calculations for PBMC freezing solution below.

Calculations for PBMC freezing solutions:

Freezing **Solution A** preparation (mL) = HuAB serum

Freezing **Solution B** preparation (mL) = HuAB serum + 20% DMSO

Total freezing solution volume used (mL) = 50% **Solution A** (HI HuAB serum)

+

50% **Solution B**

Total volume = 1.0 mL x Number of vials

NOTE: The addition of DMSO to serum is an exothermic reaction. Ensure that the DMSO:HuAB mix is at RT before use.

3. Slowly re-suspend PBMC with gentle mixing in appropriate volume of HI HuAB serum (Solution A) to make 1mL aliquots.
4. Add an equal volume of RT HuAB:DMSO mix (Solution B) dropwise with gentle mixing. The final concentration per 1 mL aliquot should not exceed 21.5×10^6 cells/mL.
5. Immediately following the addition of HuAB:DMSO mix, aliquot re-suspended PBMC mixture into 1mL aliquots in labelled 1.8mL cryovials and place in Mr. Frosty freezing container (equilibrated to RT).
6. Immediately transfer Mr. Frosty to a -80°C freezer for a minimum of 12 hours and maximum of 96 hours and then transfer to a liquid nitrogen tank for long term storage.

NOTE: It is recommended to store cryovials in vapour phase of liquid nitrogen tank; tubes in liquid phase are at risk of exploding during thawing.

8. QUALITY CONTROL / QUALITY ASSURANCE

All the equipment used should be monitored, cleaned and calibrated per their specific SOPs.

Reagents with an expiry date should be monitored and used before this date. If used after expiry date, it should be recorded.



9. APPENDICES/FORMS

9.1 Appendix A – Sample processing form: PBMC via Leucosep



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