Montreal Neurological Institute and Hospital
McGill University

CBIG-02-001

PBMC ISOLATION FROM WHOLE BLOOD (CONVENTIONAL METHOD)

Version:	1.0	Supersedes:	NA
Category:	Procedural	Effective date:	01-May-2020

Prepared By:	Marie-Noëlle Boivin	Research Assistant	
Reviewed By:	Mahdieh Tabatabaei Shafiei	Research Assistant	
	Sonia Lai Wing Sun	Operations Manager	
Approved By:	Jason Karamchandani, MD Scientific Director	Jason Karamchandani	

TABLE OF CONTENTS

Montreal Neurological Institute/Hospital, McGill University
3801 University Street, Suite NWB132/140/150
Montreal (QC), Canada, H3A 2B4
Phone: 514-398-8439
Email: cbig.mni@mcgill.ca
Website: www.mcgill.ca/c-bigneuro/

1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	01-May-2020

2. SCOPE AND APPLICATION

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

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-001 PBMC Isolation from Whole Blood (Conventional 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. GE Healthcare, Instructions 71-7167-00 AG, Ficoll-Paque Plus (February 2007)
2. Experimental Therapeutics Program: ETP-P-0001 PBMC Isolation from Whole Blood

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who process human peripheral blood samples for PBMC isolation. 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 substitutes 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 substitutes 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, 10 mL	
Disposable transfer pipets	
Pipet tips, 200 µL	
Pipet tips, 1000 µL	
Polypropylene conical tubes, 15 mL	
Polypropylene conical tubes, 50 mL	
Flip cap polypropylene conical tubes, 50 mL	ThermoScientific, Nunc EZ Flip Cap; 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	

Montreal Neurological Institute/Hospital, McGill University
3801 University Street, Suite NWB132/140/150
Montreal (QC), Canada, H3A 2B4

Phone: 514-398-8439
Email: cbig.mni@mcgill.ca
Website: www.mcgill.ca/c-bigneuro/

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 Conventional Method

Blood Volume	Processing
> 5 mL	Process in 50 mL tubes (refer to section 7.1.1 for processing)
= 4 or 5 mL	Process in 15 mL tubes (refer to section 7.1.2 for processing)

7.1.1 *In Vitro* Isolation of Human PBMC from Whole Blood (> 5 mL)

1. Distribute whole blood from any number of GTT or PTT equally into 50 mL flip cap conical tubes so that each tube contains between 10-20 mL of blood (approximately two full GTTs or PTTs per 50 mL conical tube).
2. Rinse blood collection tubes with PBS and distribute into flip cap conical tubes.
3. Dilute the blood in an approximately equal volume of PBS (1:1 ratio). The maximum volume should not exceed 30 mL. Mix gently using a serological pipet. Do not mix by inverting the tube to avoid blood accumulation in the cap and subsequent seepage during centrifugation.
4. With the pipet, carefully add 10-15mL of Ficoll (1:2 ratio Ficoll:blood and PBS mix in flip cap conical tube) to individual whole blood and PBS mixture by placing the pipet at an angle at the bottom of the 50 mL conical tube. The final volume should be between 30-45 mL prior to centrifugation.

IMPORTANT: Do not mix Ficoll and the diluted blood sample. The layers should be as distinct as possible. Do not introduce any air bubbles when adding the Ficoll layer to the diluted blood as this will have profound effect on subsequent separation.

5. Centrifuge at RT for 30 min at 700Xg with medium acceleration and without brake.

NOTE: After centrifugation, the following layers are observed:

PBS/Plasma

PBMC (also called lymphocyte layer or white "buffy coat")

Ficoll

Red blood cells

NOTE: It is critical to remove all the PBMC at the interface with minimum contamination from the Ficoll layer and the PBS/plasma layer. Removing excess Ficoll results in granulocyte contamination whereas removing excess plasma results in contamination with platelets and plasma proteins.

6. Carefully collect the PBMCs located between the PBS/plasma and Ficoll layers using a transfer pipet. Transfer into a new 50 mL conical tube.
7. Complete with PBS to 50 mL total volume immediately to dilute any Ficoll that may have been aspirated with the PBMC.
8. Centrifuge for 15 min at 480xg. Discard supernatant and keep the cell pellet.
9. Loosen 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 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 12 min at 310Xg.

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

7.1.2 *In Vitro* Isolation of Human PBMC from Whole Blood (4 or 5 mL)

1. Transfer whole blood from GTT or PTT into 15 mL conical tube.
2. Rinse blood collection tubes with PBS and pour into conical tubes.
3. Dilute blood in a total equal volume of PBS (1:1 ratio). The maximum volume should not exceed 10 mL.
4. Mix gently using a serological pipet. Do not mix by inverting the tube to avoid blood accumulation in the cap and subsequent seepage during centrifugation.
5. In a new 15 mL conical tube, add Ficoll (1:2 ratio Ficoll:blood and PBS mix).
6. Gently overlay diluted blood on top of Ficoll solution.
7. Follow steps 5 to 11 of section 7.1.1.

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

7.2 Summary Table

Steps	
Blood volume > 5mL	**4mL ≤ Blood volume ≥ 5mL**
1. Distribute between 10-20 mL of blood into 50 mL conical tubes	1. Transfer blood into 15 mL conical tube
2. Dilute blood with equal volume of PBS	2. Dilute blood with equal volume of PBS
3. Add Ficoll (1:2 ratio with volume of blood/PBS) below the blood/PBS	3. Add Ficoll to a new 15 mL tube (1:2 ratio with volume of blood/PBS)
4. Centrifuge for 30 min at 700Xg	
5. Collect PBMC layer and transfer to a new 50 mL conical tube, complete volume to 50 mL with PBS	
6. Centrifuge for 15 min at 480Xg and discard supernatant	
7. Loosen cell pellets and pool them in a final volume of 10 mL PBS (count cells if needed)	
8. Complete to 50 mL total volume with PBS and centrifuge for 12 min at 310Xg	
9. 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 1 mL 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 $21.5x10^6$ cells/mL.
5. Immediately following the addition of HuAB:DMSO mix, aliquot re-suspended PBMC mixture into 1 mL aliquots in labelled 1.8 mL 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 as 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 (Conventional Method)

Montreal Neurological Institute/Hospital, McGill University
3801 University Street, Suite NWB132/140/150
Montreal (QC), Canada, H3A 2B4

Phone: 514-398-8439
Email: cbig.mni@mcgill.ca
Website: www.mcgill.ca/c-bigneuro/