

Isolating PBMCs from Vacutainer® Tubes	Revision Number: 03
	Effective Date: 12Mar25
	Written/Revised By: Wendy Runyon

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Version Number	Summary of Changes	Revised by/Date
01	Original Document	Daeun Jeong 16April2016
02	Updated cryopreservation medium, standardized all centrifugation speeds to RCF, changed pipetting instructions, added Vacutainer® figure	Wendy Runyon 25May2023
03	Updated "SOP Number" to "Revision Number". Added cryovials and freezing container to Supplies section	Wendy Runyon 12Mar25

Note: Please acknowledge the Gladstone Stem Cell Core in your presentations and publications if you have used the core's infrastructure (lab space or equipment), reagents/media, expertise, and/or services. These acknowledgments help to justify the existence of the core facility, as well as allowing Gladstone to be more competitive in grants and pursue other resource opportunities in order to enhance the cores over time.

1. PURPOSE

To describe the procedure for isolating and cryopreserving PBMCs from whole blood collected in Vacutainer® CPT™ tubes for the purpose of iPSC reprogramming.

2. SUPPLIES

BD Vacutainer® CPT™ tubes with Sodium Citrate (362761, BD)
 IMDM (12440053, Life Technologies)
 CryoStor® CS10 (210102, Biolife Solutions)
 Cryovials
 Controlled rate freezing container (such as Mr. Frosty or Freeze Cube)

3. PROCEDURE

3.1. Obtain Vacutainer® CPT™ tube(s) containing whole blood.

Note: Vacutainer® tubes should be stored upright at room temperature and processed as soon as possible. For best results, samples should be centrifuged within two hours of collection. Time between blood collection and processing/cryopreservation should not exceed 24 hours.

3.2. Remix the blood sample by gently inverting 8-10 times.

3.3. Centrifuge the Vacutainer® tube(s) at room temperature in a horizontal rotor (swing-out head) for 20 minutes at 1600 RCF. Ensure placement of the tubes in the centrifuge adapter will not cause tubes to break when the swinging bucket rotates.

Note: Adjust the centrifuge braking speed for approximately 2 minutes of braking time.

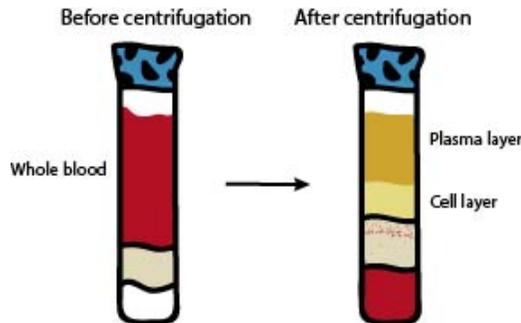
3.4. Remove the plasma layer without disturbing the cell layer (see figure below).

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- 3.5. Transfer the cell layer to a 15mL conical, making sure to pipette cells from the sides of the tube. Repeat for any additional tubes from the same donor and add to the same 15mL conical.
- 3.6. Add cold IMDM to the 15mL conical containing the cell suspension to bring the total volume in the conical to 12-15mL.
- 3.7. Centrifuge at room temperature for 5 minutes at 300 RCF.
- 3.8. Remove supernatant and resuspend the cell pellet by finger tap.
- 3.9. Add 10mL cold IMDM and pipette 3-5 times to mix. Perform cell count.
- 3.10. Centrifuge at room temperature for 5 minutes at 300 RCF.
- 3.11. Remove supernatant and resuspend the cell pellet by finger tap.
- 3.12. Add an appropriate volume of CryoStor for a concentration of 4-5e6 cells/vial.

$$\text{Total Cells } (V_{(\text{Total})} \times C_{(\text{live cells/mL})}) \div C_{(\text{Cells/vial})} = V_{\text{CryoStor}}(\text{mL})$$

- 3.13. Dispense 1mL cell suspension into pre-labeled cryovials and place in a controlled rate freezing container, such as a Mr. Frosty.

Note: Cryovials should be labeled with “PBMCs”, de-identified cell line name, date, total number of cells, and operator initials. Do not include donor information on the label.

- 3.14. Store the cells in the controlled rate freezing container at -80°C overnight and transfer to liquid nitrogen storage the following day. Keep vials on dry ice while transporting to liquid nitrogen storage and transfer vials as quickly as possible to prevent warming.