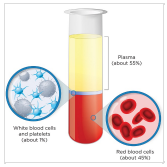

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PBMC Isolation

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Isolation of peripheral blood monocytes (PBMC) for later expansion and differentiation

PROTOCOL CITATION

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MATERIALS

NAME	CATALOG #	VENDOR
Tricine		P212121
Sodium Chloride	PubChem CID: 5234	
Hydrochloric Acid		
Fetal Bovine Serum	10270106	Gibco - Thermo Fischer
Molecular Biology Grade Water	10154604	Fisher Scientific
CryoStor® CS10 100 mL Immunology	7956	Stemcell Technologies
Sodium Hydroxide		
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
Mr. Frosty Freezing Container, 2mL tubes, Nalgene Mr. Frosty Freezing Container for 1-2mL cryogenic tubes, PC, clear w/ blue lid, 1/Cs.	5100-0001	Thermo Fisher
OptiPrep™ Density Gradient Medium	D1556	Sigma Aldrich
Denville Posi-click 1.7ml Eppendorf Tubes	C2172	
BD Vacutainer™ Plastic Blood Collection Tubes with K2EDTA: Hemogard™ Closure	BD 367862	Fisher Scientific
Molecular grade water	BP561-1 1L	

Solution Preparation

1 Prepare [M]**100 Milimolar (mM)** Tricine Stock **(Fresh)**

- 0.895g Tricine

- 50ml Water

2 Prepare Buffered Saline Solution **(Solution B)** **Room temperature**

- Dissolve 0.85g of NaCl in 50 ml water
- Add 10 ml of Tricine stock solution
- Adjust to pH 7.0 with 1 M NaOH pH **7**
- Volumize to 100 ml.

3 Prepare Density Barrier **Room temperature**

- Dilute Solution B with molecular grade water (volume ratio 2.5:0.5 respectively)
- Check osmolality of the solution - **should be 242 +/- 10**
- Dilute OptiPrepTM with this solution using a volume ratio 2.7: 9.3 respectively
- Sterile filter in a hood before use

* See Barrier Solution calculation sheet to determine the quantity needed for the number of animals being harvested

4 Prepare Solution B + 2% Fetal Bovine Serum **Room temperature**

- Mix 50ml Solution B + 1ml FBS
- Sterile filter in a hood before use

Blood Collection

5
- Collect blood by cardiac puncture into a 10ml syringe with 18g needle by slowly allowing blood to enter the syringe.
- Remove needle and drop blood into a purple top EDTA Tube (**no vacuum – this increases hemolysis**). Hemolysis can increase the "stickiness" of the PBMCs, avoid this and clotting for the cleanest preparations.
- 4ml collections into a single tube are most effective – **the ratio of EDTA to blood is important** – smaller volumes of blood do not separate as well and yield far less cells.

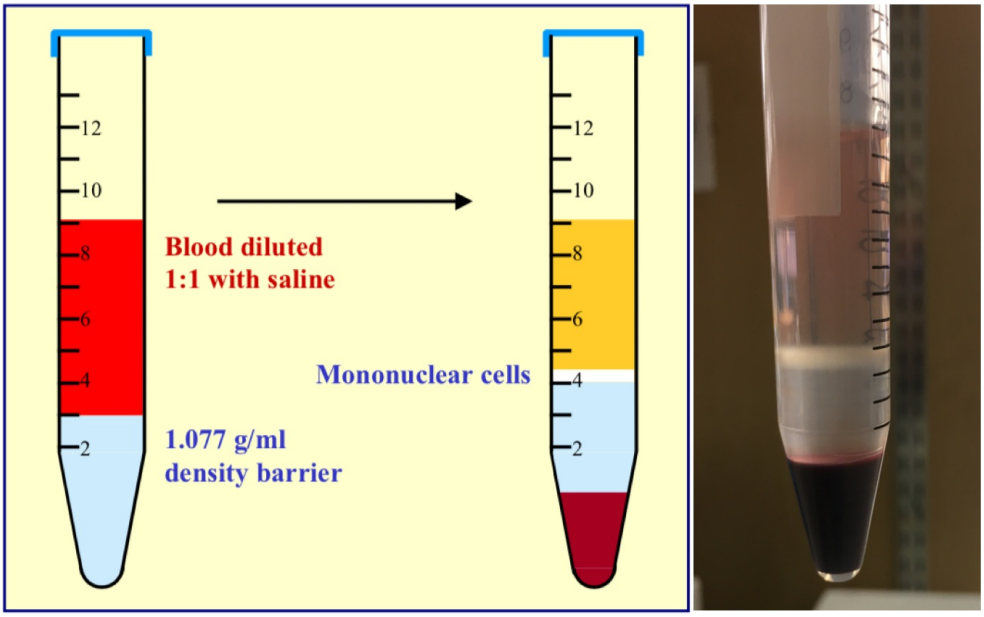
PBMC Isolation 45m

6
- In a cell culture hood - dilute the blood (2ml) with an equal volume of Solution B + 2% FBS.
- Save 2x500ul aliquots of FBS used as a control for later experiments.
- **FBS is essential to allow PBMC to not stick** to lower layer of density gradient, otherwise cells are much harder to isolate and there are significant numbers of RBCs that are difficult to wash out.

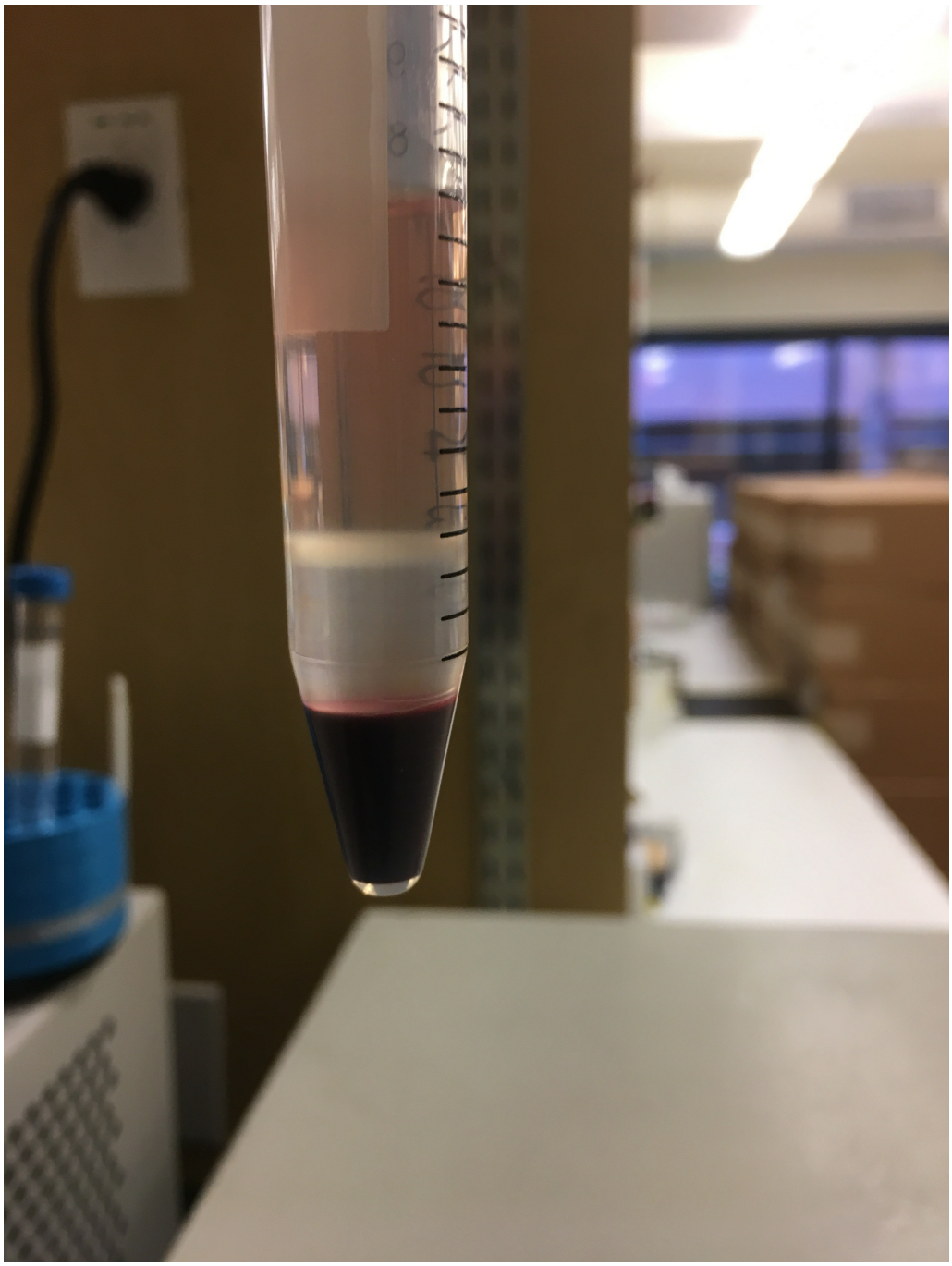
7
- In a cell culture hood - carefully layer 4 ml of diluted blood over 2 ml of the **Density barrier** in a 15ml Falcon Tube
- Maintain a 2:1 ratio of blood to density barrier if there is more blood available - eg. 6ml diluted blood to 3ml barrier
- **(SUPER IMPORTANT- avoid mixing at the interface –SUPER IMPORTANT)**
- This requires a slow and steady hand to carefully pipet the blood over the density barrier. Take your time!

8 Centrifuge at 700 g for 30 min at **Room temperature** in a swinging-bucket rotor with slowest acceleration and deceleration with **no brake**. 45m

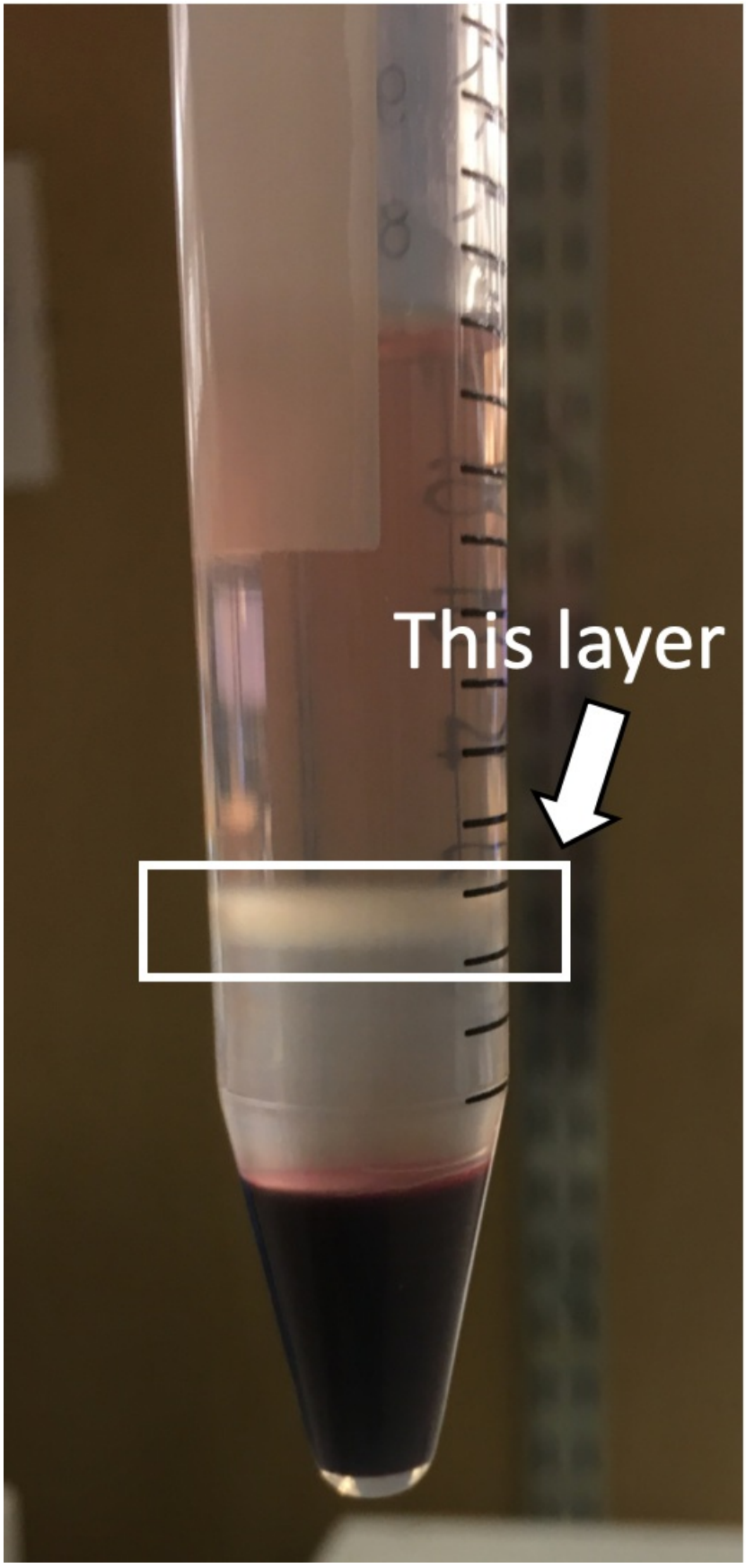
- Do not use 4C it will not separate
- Do not use the brake as it will disrupt the formation of the layers.
- It will take ~10 min for the rotor to stop spinning after the centrifugation step as there is no brake.



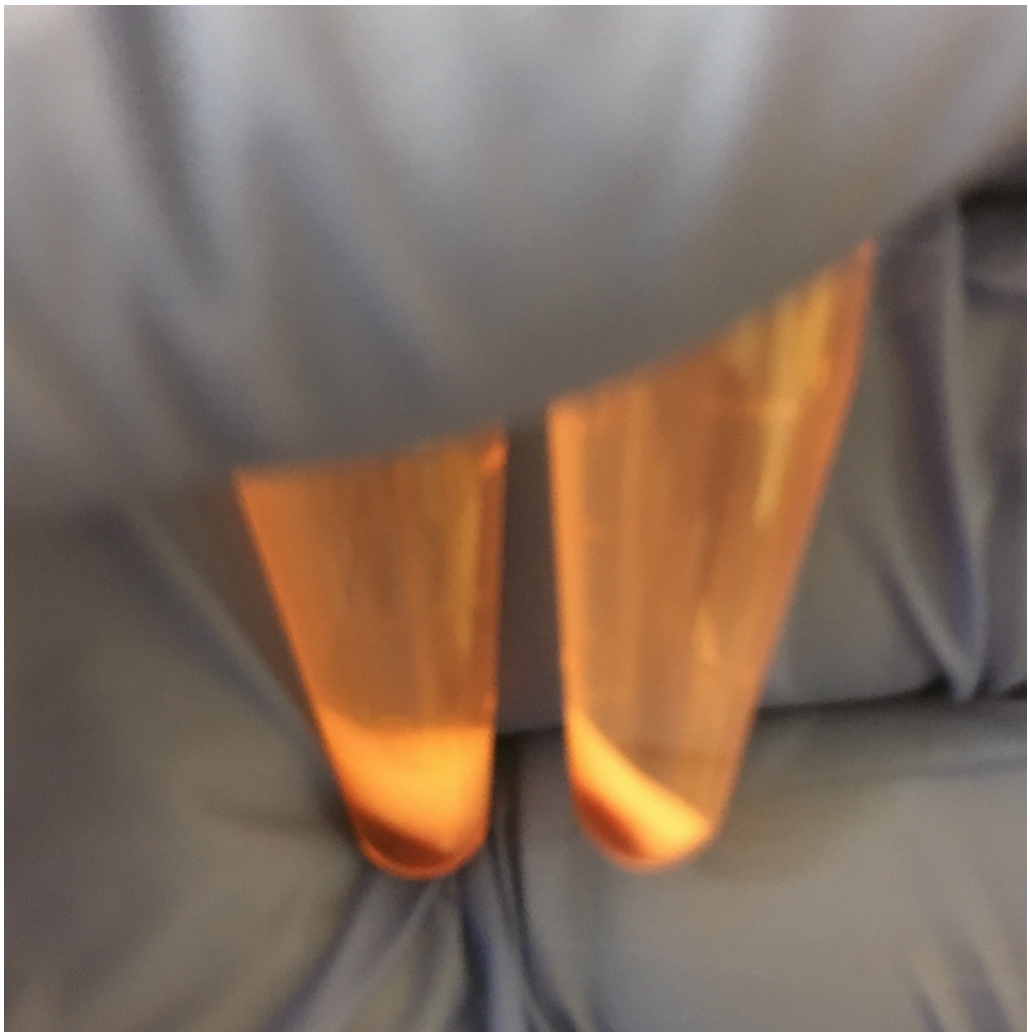
9 After centrifugation the PBMCs form a sharp band at the interface.



10 Gently insert the pipet tip and extract the PBMC layer. ~100-200ul depending on how sticky they are to the lower layer. Sometimes there is really nice separation, but sometimes the PBMCs are stuck directly to the blood layer below. Avoid picking up the erythrocytes if possible.



11 Dilute by 2X in Solution B + 2% FBS and pellet the cells at 400g for 10 min to wash (X2).



PBMCs in white, leftover erythorocytes in red. Avoid picking up red blood cells when pipetting in the next step.

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- Resuspend the PBMC pellet in Cytostor10 (250ul) and take note of pellet size 0-3
- Transfer aliquot to a white top cryotube with appropriate labeling

Place resuspended cells in Mr. Frosty o/n to reach -80 for at least 24h, at which point the cells can be transferred to

13 the liquid nitrogen. Make sure the Mr. Frosty has enough isopropanol in the bottom container to reach the line labeled on the side.