

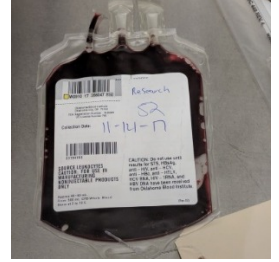
Jun 10, 2018 Version 2

## 🌐 PBMC isolation from buffy coat V.2

📁 In 5 collections

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** June 10, 2018

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**Protocol Integer ID:** 12922


## Materials


### MATERIALS


 Corning™ cellgro™ Lymphocyte Separation Medium **Fisher Scientific Catalog #MT25072CV**

 Countess™ Cell Counting Chamber Slides **Catalog #C10314**

 PBS Tablets, Phosphate Buffered Saline, Fisher BioReagents **Fisher Scientific Catalog #BP2944100**

 Fisher BioReagents™ Bovine Serum Albumin (BSA) Protease-free Powder **Fisher Scientific Catalog #BP9703100**

 Ethylenediaminetetraacetic Acid (0.5M Solution/pH 8.0), Fisher BioReagents **Fisher Scientific Catalog #BP2482-500**


 EMD Millipore™ Stericup™ Sterile Vacuum Filter Units **Fisher Scientific Catalog #SCGPU05RE**



**1 Prepare isolation buffer:**

- 500 ml PBS w/o Mg and Ca (Fisher Bioreagents, BP2944-100)
- 0.5 g BSA (Fisher Scientific, BP9703-100)
- 2 ml 0.5 M EDTA (Fisher Scientific, BP2482500)

Mix them all and sterile filter the buffer (EMD Millipore™ Stericup™ Sterile Vacuum Filter Units, 0.22 um). Keep it in the fridge.

- 2 Fill 4× 50-ml falcons with 15 ml isolation buffer.
- 3 Cut the buffy coat and fill another 50 ml falcon tube.
- 4 Split the 50 ml blood between 4X 50-ml falcons that have the isolation buffer.
- 5 All tubes should have ~30 ml blood/buffer mix at this point.
- 6 Using a 10 ml pipet, gently underlay 14 ml of Corning™ cellgro™ Lymphocyte Separation Medium below the blood.
- 7 Centrifuge at 800 X g for 25 min at RT with soft deceleration.
- 8 In the meantime, get 4 new 50 ml falcons.
- 9 After the centrifuge, use a 10 ml pipet to transfer the cloudy buffy layer to a fresh tube.
- 10 Wait for 1-2 mins for more of the buffy layer to form and transfer that to the fresh tube as well.  
 00:02:00
- 11 Add cold isolation buffer to each tube so that the final volume is now 40 ml.
- 12 Mix by gently inverting tubes for a few times and centrifuge at 500 X g for 10 min at 4°C.

- 13 Discard the supernatant (leave ~5ml liquid) into a bleach filled container.
- 14 Combine the pellet from all tubes into one tube and increase the volume to 40 ml by adding isolation buffer.

### Count the cells (1:10 dilution)

- 15 Take 10 ul from the sample and mix with 90 ul of PBS (1:10)
- 16 Mix with 10 ul of Trypan Blue with 10 ul of the sample and load 10 ul to the cell counter chamber for counting.

#### Equipment

<b>new equipment</b>	NAME
Thermo Fisher Scientific	BRAND
AMQAX1000	SKU
Countess™ II Automated Cell Counter	SPECIFICATIONS

- 17 Continue with the desired T cell isolation kit or freeze PBMC cells.