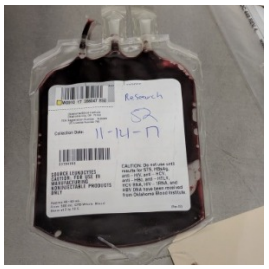
Jun 10, 2018 Version 2

PBMC isolation from buffy coat V.2



In 5 collections

DOI
dx.doi.org/10.17504/protocols.io.qu2dwye

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.qu2dwye

Protocol Citation: Bulent Arman Aksoy, Pınar Aksoy, Megan Wyatt, Chrystal M. Paulos, Jeff Hammerbacher 2018. PBMC isolation from buffy coat. **protocols.io https://dx.doi.org/10.17504/protocols.io.qu2dwye**

Protocol status: Working
We use this protocol and it's working

Created: June 10, 2018

Last Modified: June 10, 2018

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

new equipment	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.