VERSION 1

JUN 10, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocol.s.io.quzdw6

Protocol Citation: Bulent Arman Aksoy, Pinar Aksoy, Megan Wyatt, Chrystal M. Paulos, Jeff Hammerbacher 2018. PBMC isolation from buffy coat. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.quzdw6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jun 10, 2018

Last Modified: Jun 10, 2018

PROTOCOL integer ID:
 12921

🌐 PBMC isolation from buffy coat V.1

Bulent Arman Aksoy¹, Pinar Aksoy¹, Megan Wyatt¹, Chrystal M. Paulos¹, Jeff Hammerbacher¹

¹Medical University of South Carolina

Bulent Arman Aksoy
 Medical University of South Carolina

MATERIALS

MATERIALS

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

Countess™ Cell Counting Chamber Slides **Contributed by users Catalog**
#C10314

1 Fill 4x 50-ml falcons with 15 ml isolation buffer.

- 2 Cut the buffy coat and fill another 50 ml falcon tube.
- 3 Split the 50 ml blood between 4X 50-ml falcons that have the isolation buffer.
- 4 All tubes should have ~30 ml blood/buffer mix at this point.
- 5 Using a 10 ml pipet, gently underlay 14 ml of Corning™ cellgro™ Lymphocyte Separation Medium below the blood.
- 6 Centrifuge at 800 X g for 25 min at RT with soft deceleration.
- 7 In the meantime, get 4 new 50 ml falcons.
- 8 After the centrifuge, use a 10 ml pipet to transfer the cloudy buffy layer to a fresh tube.
- 9 Wait for 1-2 mins for more of the buffy layer to form and transfer that to the fresh tube as well.

- 10

Add cold isolation buffer to each tube so that the final volume is now 40 ml.
- 11

Mix by gently inverting tubes for a few times and centrifuge at 500 X g for 10 min at 4°C.
- 12

Discard the supernatant (leave ~5ml liquid) into a bleach filled container.
- 13

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)

- 14

Take 10 ul from the sample and mix with 90 ul of PBS (1:10)
- 15

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	
	NAME
Thermo Fisher Scientific	BRAND
AMQAX1000	SKU
Countess™ II Automated Cell Counter	SPECIFICATIONS

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