PBMC isolation from buffy coat

Bulent Arman Aksoy,Pınar Aksoy,Megan Wyatt,Chrystal M. Paulos,Jeff Hammerbacher

Abstract

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Materials

Corning™ cellgro™ Lymphocyte Separation Medium MT25072CV by Fisher Scientific

Countess™ Cell Counting Chamber Slides C10314 by Contributed by users

Protocol

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

Step 2.
Cut the buffy coat and fill another 50 ml falcon tube.

Step 3.
Split the 50 ml blood between 4X 50-ml falcons that have the isolation buffer.

Step 4.
All tubes should have 30 ml blood/buffer mix at this point.

Step 5.
Using a 10 ml pipet, gently underlay 14 ml of Corning™ cellgro™ Lymphocyte Separation Medium below the blood.

Step 6.
Centrifuge at 800 X g for 25 min at RT with soft deceleration.

Step 7.
In the meantime, get 4 new 50 ml falcons.

Step 8.
After the centrifuge, use a 10 ml pipet to transfer the cloudy buffy layer to a fresh tube.

Step 9.
Wait for 1-2 mins for more of the buffy layer to form and transfer that to the fresh tube as well.

Step 10.
Add cold isolation buffer to each tube so that the final volume is now 40 ml.

Step 11.
Mix by gently inverting tubes for a few times and centrifuge at 500 X g for 10 min at 4°C.

Step 12.
Discard the supernatant (leave 5ml liquid) into a bleach filled container.

Step 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)

Step 14.
Take 10 ul from the sample and mix with 90 ul of PBS (1:10)

Count the cells (1:10 dilution)

Step 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
Equipment brand:
Thermo Fisher Scientific
SKU:
AMQAX1000
Specifications:
Countess™ II Automated Cell Counter

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