Isolation of leucocytes from human blood

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Abstract

Protocol established by Anna F. Zetler and published by Luisa F. Jiménez-Soto

This protocol is the final adaptation of protocols used in the laboratory of Prof. Rainer haas. The following people contributed with their ideas and experience: Benjamin Busch, Bettina Vogl-Gebert and Luisa F. Jiménez-Soto

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Protocol

Extract human blood

Step 1.

Following ethical regulations and hygiene standards, whole blood was drawn from human volunteers into a collection tube containing at least 20u/ml of Heparin.

Prepare blood for gradient centrifugation

Step 2.

- Dilute blood (12ml whole blood and 25 ml PBS/2mM EDTA, Mix by inversion).
- Add 35 ml of the diluted blood carefully onto 15 ml of Percoll™ Plus 55% isotonic (GE Healthcare Life Science). Avoid mixing cells and Percoll® Plus.

REAGENTS

Percoll™ Plus 17-5445-01 by Ge Healthcare

Centrifuge to separate the leucocytes from erythrocytes

Step 3.

Centrifuge the gradient in a swing rotor centrifuge at 400g for 20 minutes at 16°C without brake.

Extract leucocytes from gradient

Step 4.

At the end of centrifugation, remove carefully the tube from the rotor. You should see the formation of three layers: i) Serum + Platelets; ii) Leucocytes, and iii) Erythrocytes.

Wash, collect and prepare leucocytes.

Step 5.

- Centrifuge the leucocyte solution in a swing-rotor centrifuge at 200 g for 5 min 16°C.
- Remove supernatant and repeat twice with PBS to remove any trace of Percoll Plus
- Resuspend cells in RPMI 1640 media complemented with 10% FBS.
- Count cells using Trypan Blue for viability count and seed on cell culture treated plates as needed ($1x10^6$cells per well usually).

Incubate cells

Step 6.

Place cells at 37°C in a 5% CO_2 atmosphere. We used the cells in the next 24 hours to secure a close physiological behaviour.