EXPERIMENTAL PROTOCOL: Isolation of PBMCs by Ficoll-Paque density gradient centrifugation

Author/s: Susana Torres Martínez and Andrea Moreno Manuel
Contact information: susana.torres@goumh.umh.es;
andrea.morenomanuel@gmail.com

Introduction

Ficoll-Paque can be used to isolate lymphocytes from blood, bone marrow and umbilical cord blood through density gradient centrifugation. Since the densities of mononuclear cells such as lymphocytes and monocytes are less than that of Ficoll-Paque (1.077 g/ml), these cells create a layer at the interphase during density gradient centrifugation, and therefore can be readily collected.

Reagents

- Ficoll-Paque™ PLUS (Ref. 11778538)
- Phosphate Buffered Saline w/o Calcium w/o Magnesium (PBS)
- RNAlater® Stabilization Solution
- Fetal Bovine Serum (FBS)
- Dimethyl sulfoxide (DMSO)
- Trypan Blue Solution, 0.4%

Instruments and tools

- Laminar flow cabinet
- Centrifuge with swing-bucket rotors and acceleration and deceleration (braking) ramps
- Anticoagulated blood tube (e.g. BD Vacutainer® EDTA Tubes)
- Sterile 15 ml conical tubes
- 1,5ml microtubes
- LDPE Pasteur Pipettes
- Glass Pasteur Pipettes
- Rubber Small Volume Pacifier
- Cryogenic Vials

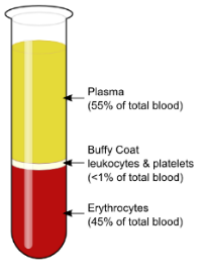
- Freezing containers
- Neubauer chamber
- Micropipettes

Before start

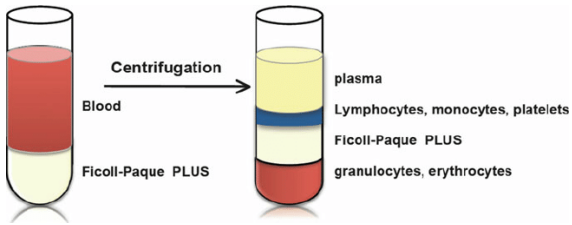
- All steps should be performed on a laminar flow cabinet to prevent contamination.
- There are several Ficoll-Paque media available, and users should decide which to use depending on the species and the cells needed. Ficoll-Plaque PLUS (1.077 g/ml) is widely used for the isolation of human mononuclear cells, and we have had success with it to isolate lymphocytes and monocytes from human blood samples.
- Depending on the further procedure, different anticoagulant blood tubes may be chosen, such as EDTA, citrate or heparin.

PROCEDURE

1. Collect blood on BD Vacutainer® EDTA Tubes (2 tubes).
2. Mark the level of blood on EDTA Tubes.
3. Centrifuge EDTA tubes at 360g for 20min at 20ºC.
4. Transfer plasma fraction into a 15mL conical tube, leaving 15% of plasma in the EDTA tube to be sure that all leukocytes remain.



5. Dilute blood with sterile PBS up to the mark and slowly move to mix.
6. Place slowly 4mL of Ficoll-Paque™ PLUS into two sterile 15 ml conical tubes.
7. Slowly and gently release a maximum of 5ml of blood on each falcon tube with Ficoll-Paque™ PLUS without disturbing Ficoll interphase with a LDPE Pasteur Pipette.
8. Weigh samples to ensure proper balance of the rotors.
9. Centrifuge at 400g for 30 min at 20ºC with slow acceleration and brakes turned off.
10. Carefully remove the tube from the rotor to avoid any disturbance to the layers formed during centrifugation.



11. Using a sterile glass pasteur pipette, transfer the layer of mononuclear cells into a clean sterile labeled 15ml conical tube, along with the remaining media above the Ficoll-Paque. Minimize transfer of the Ficoll-Paque.
12. Wash the mononuclear cells with 8 ml of PBS (one tube for each Ficoll tube).
13. Centrifuge at 400g for 5 min at 20ºC with slow acceleration and brakes turned off.
14. Discard the supernatant, and gently resuspend cells in 2 ml of PBS and proceed with cell counting.
15. Centrifuge at 400g for 5 min at 20ºC with slow acceleration and brakes turned off.
16. Remove PBS and depending on downstream application resuspend the cells accordingly.
    16.1. If cells will be used for RNA extraction, resuspend cells with RNA later (2 volumes of RNA later) and divide them into two 1.5ml microtubes. Tubes are kept in -80ºC freezer.
    16.2. If cells are to be frozen for further culture, resuspend cells in freshly made freezing media of 90% FBS and 10% DMSO (3-4 million of cells/ml of freezing media). Transfer to sterile labeled cryovials. Cryovials are kept in a freezing container (i.e. Mr. Frosty containing fresh isopropyl alcohol) in −80ºC freezer overnight prior to storage in liquid nitrogen.