

SOP-P032

Ficoll-Hypaque: Overlay Method

Objective: To isolate lymphocytes from whole blood for further immunological studies such as immunophenotyping

Procedure:

1. Dilute one volume of anticoagulated peripheral blood with two volumes of Phosphate Buffered Saline (PBS). See SOP#P020 for preparation of PBS.
2. Ficoll Hypaque is stored at 4°, so you must let it warm to room temperature before use.
3. Using a serological pipet, transfer 4ml of ficoll into each 15cc conical tube.
4. Using a new serological pipet, aspirate 8ml of the diluted whole blood.
5. Holding the 15cc conical tube with the ficoll in it at an angle, very slowly and gently layer the 8ml of diluted whole blood on top of the ficoll by placing the tip of the pipet close to the blood and gently and slowly dispense the blood as you simultaneously pull the tip of the pipet up. It is important not to cause any turbulence at the blood/ficoll interface.
6. Place the lid on the 15cc conical tube.
7. Centrifuge at 400xg for 30minutes at room temperature.
8. When removing the 15cc conical tube from the centrifuge be careful not to disrupt the layers. You should see the red blood cells at the bottom. The granulocytes will form a slight layer directly on top of the red blood cells. The next layer will be the the "band" of mononuclear cells. On top of these cells will be the platelets, plasma and buffer combined.
9. Using a pasteur pipet, gently remove the plasma/buffer layer and discard.
10. Using a clean pasteur pipet aspirate off the mononuclear (lymphs) layer and transfer to a new 15cc conical tube.
11. Wash the cells by adding 10ml of PBS and centrifuging at 200xg for 10minutes.
12. Decant and repeat wash.
13. Resuspend the cells in a known volume of PBS.
14. Do a cell count on the Coulter Counter. See SOP#P005 for operation of the Coulter Counter.
15. Adjust to desired cell concentration.

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DATE: 2/23/99

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