SOP-P033

Ficoll-Hypaque: Underlay 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 8ml of diluted whole blood into each 15cc conical tube.

4. Using a new serological pipet, aspirate 4ml of the ficoll.

5. Carefully underlay the ficoll by gently putting the tip of the pipet at the bottom of the 15cc conical tube underneath the diluted whole blood. Gently and slowly dispense the ficoll, it should push up the blood. Be careful not to cause turbulance at the ficoll/blood 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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Verified by:_____ Date:_____ _____

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