

PREPARATION OF BUFFY COAT (Cheon Fai Lai)

Collect blood in Heparinized tube @ 100 mu/10 ml.

Add 1:1.5 vol of 3% Dextran in the medium to be used.

Sediment for 30-45 minutes at room temperature.

Aspirate supernatant and dilute 1:1.5 with medium.

Centrifuge @ 400g for 10 minutes.

To eliminate RBC's, use hypotonic shock

Resuspend cells in 1 ml medium and add 2.5 vol. of dist. H₂O

Shake vigorously for up to 20 seconds (not greater than 20 seconds).

Add 2.5 volumes of 2x medium.

Add 6x volume of 1x medium and layer over FCS to eliminate ghosts.