Isolating primary B cells from human blood

Using Miltenyi Biotec Human B cell Isolation Kit II MACS
(Order NO. 130-091-151)

<Isolation of PBMC (Peripheral blood mononuclear cells)using Ficoll Paque>

1. start with freshly drawn human blood not older than 8 hours, treated with an anti-coagulant (use 500ul of 2.7% EDTA for 10ml blood if you are not using EDTA coated tubes when collecting blood)
2. dilute cells with 4 volumes of PBS/EDTA
3. carefully layer 35 ml of diluted cell suspension over 15ml Ficoll Paque (1.077 density) in a 50 ml conical tube and spin at 400g for 40min at 20C in a swinging bucket rotor without brake
4. aspirate upper layer, leaving the mononuclear cell layer undisturbed at the interphase (I aspirate the upper layer until I get 5mm close to the interphase, and take all the interphase until I get 5mm close to red blood cells. Collecting as much as you could will improve the recovery. It is O.K. to have Ficoll in your cells, as long as it is mixed well.)
5. carefully transfer the interphase (monocytes, thrombocytes, and lymphocytes) (granulocytes , which is the majority of the white blood cells,will pass through the Ficoll) into a new 50ml tube.
6. fill the conical tube with PBS/EDTA/FBS, mix and centrifuge at 300g for 10’ at 20C. Remove the supernatant.
7. for removal of platelets, resuspend the cell pellet in 1ml PBS/EDTA/FBS, take out 10ul and count number of cells
8. dilute cells in 35ml PBS/EDTA/FBS and carefully lay it over 15ml of solution with density 1.063 , centri at 350g 15min 20C . Mononuclear cells will pass through and platelets will remain in the supernatant.
9. aspirate the media and resuspend cell pellet in 1ml PBS/EDTA/FBS, take 10ul to count cells.
10. PBMCs may be stored at 4C for o/n in PBS/EDTA/FBS. Do not store cells longer than 1 day. Wash at least once before proceeding to magnetic labeling.

<Magnetic labeling>

Reserve magnet from the flow lab in the hospital, they have VarioMACS
Work fast, keep cells cold, use pre-colled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

1. determine cell number, if more than 1*10^7 cells, scale up all reagent vol.
2. spin down cells at 300g for 10min. remove supernatant completely. (The affinity of antibody is maximized at this condition. So try to keep vol correct)
3. resuspend cell pellet in 40ul of PBS/EDTA/FBS per 10^7 total cells
4. add 10ul of biotin-antibody cocktail
5. mix well and incubate for 10min at 4C
6. add 30ul of PBS/EDTA/FBS
7. add 20ul of anti-biotin microbeads
8. mix well and incubate for an additional 10min at 4C
9. Prepare column (LS column) by rinsing with 3ml PBS/EDTA/FBS
10. apply cell suspension onto the column
11. allow the cells to pass through and collect effluent (enriched B cell fraction)

12. wash column with 3*3ml PBS/EDTA/FBS
13. Collect entire effluent in the same tube as step 11, this fraction represents the enriched B cells
14. (Optional) elute retained cells outside of the magnetic field. (non B cells)

* PBS/EDTA : PBS pH 7.2 , 2mM EDTA -> filter
PBS/EDTA/FBS : PBS/EDTA + 5% FBS

<Count white blood cells from blood>
Dilute blood in 20 fold PBS/EDTA
Mix blood 1:1 in lysis solution

Mix blood 1:1 in lysis solution	acetic acid	2ml
	1% crystal violet	1ml
	H_2O	100ml

This solution will stain white blood cells and lyze red blood cells.