Cell enrichment by adherence

1. Extracted total splenocytes by using a wire mesh and a scraper.
2. No spinning involved. Plate as is on 6-well plate or flask with sufficient amount of media.
3. Incubate in 27ºC over night
4. The following day, observe the plate under microscope. You will be able to see three layers or cells. The top layer would be the most visible, abundant and aggregated layer. Most of these are leukocytes and RBCs. The second layer would be not as bright so you have to look carefully to identify cells. Some of the stuff you find here are debris from the spleen. The third layer is the hardest to find. This is the layer you want. Since the cells have adhered, they will look flat, most of the time transparent and fewer cells (~1-2% of total cells per well).
5. Carefully remove the media by tilting the flask and positioning the pipette tip to the corner. Try not to agitate the flask too much. Save this fraction.
6. Carefully add cold, sterile APBS (along the flask line) to the flask and incubate the flask on ice for 1 hr. Make sure the bottom of the flask/plate is well covered with ice.
7. Tap the flask (as if you are getting cells out post trypsinase) and collect your cells by adding APBS. Wash the flask well, pipette up and down many times to prevent cell aggregation.
8. Save this fraction and check the flask/plate under the microscope. If you see cells, proceed to step 9.
9. Add 1-2mL of APBS to the flask. Place the flask on ice for 15-20 min, and scrape off the cells, very gently, with a cell scraper.
10. Add more APBS and collect the cell fraction.
11. Proceed to histology/staining.
12. If you need to culture these fraction, proceed to culturing immediately. (DC's are already active by now – just a noteworthy point). For smaller cell numbers, use a 96-well plate (flat bottom).