**MACS Protocol using CD34+-binding magnetic beads (for PBSC 34+ Sample)**

*A rough draft recorded on Howard’s words*

*August 2013*

***MACS buffer***: HBBS + 2%FBS (20mLFBS+100ml HBSS10x top up to 1000mL ddH2O)

1. Prepare 10ml MACS buffer per sample. [Eg if 4 samples, thus 40mL. #Make sure waterbath level enough water to thaw cell. (topup)]
2. Wash with MACS buffer. Centrifuge and withdraw the supernatant.
3. Add in 2mL MACS buffer & 10uL DNAse into each falcon.
4. Wait till clump dissolve.
5. Count cells (Use > volume 490uL MACS instead of 90uL to count cell)
6. Sample go through sieve (strainer)
7. Pour ~40mL MACS in the previous falcon then only to the new falcon through sieve.

* DO NOT pipette up and down the cell-> will stress the cells.

1. Centrifuge and withdraw supernatant.

#Beads may sink to bottom, thus need to shake before use.

1. ***For normal bone marrow samples (PBSC),*** 300uL MACS Buffer (first)+ 100 uL blocking buffer (added 2nd) + 100 uL beads for 100 x 10^6 cells (final) (1-5% CD34+)-> (final volume = 500 uL); (+20uL DNAse if needed)  
   ***\*For AML, 100 uL blocking buffer + 100 uL beads per 5M CD34+ cells***
2. Stain in 50 mL Falcon conical tube, at 4C room, in dark with rocking, for 50 mins – 1 hr.
3. Meantime, prepare:

* MACS magnet
* 4 x 50ml Falcon (Waste)- to put under the magnet
* 4 x 15ml Falcon (Final collection)

# **those trapped in the carbon syringe is those CD34+ wanted**

1. Fill up the collection tube 15ml Falcon with 5mL MACS.
2. After 1 hr, add in MACS buffer (~30ml) in to 4C 50ml Falcon
3. Centrifuge and withdraw the supernatant.
4. Equilibrate the LS column with 3 mL de-gased MACS buffer through strainer **before putting the cell** (40 µm) by gravity **(can ignore de-gas).**
5. Resuspend cells in 3 – 5 mL MACS buffer, and pass it through strainer and column 1ml each time. (First add 1mL MACS to the pellet in the spinned falcon) then go through the sieve. After that, use 2mL MACS to wash the balance from previous falcon and pipette the solution through the sieve.
6. Wash with 3ml MACS, 3 times. (DO NOT let the column dry totally only add)
7. Elution: Take the column away from the magnet, and replace with a new 15 mL falcon tube; put in 3 mL MACS with a plunger, push quickly, forcefully and carefully, once only into the 15ml Falcon tube.
8. Repeat the previous process with the eluent without a strainer but a new column.
9. Centrifuge at 1500rpm 5mins and withdraw supernatant.
10. Prepare the Master mix IMEM medium with cytokine. (IL3, IL6, GCSF, SCF, FLT3C)
11. Put in 1mL of the master mix medium.
12. Count cell. Top up to 8mL or appropriate amount to get 1M/mL-> Then dispense cell into the 96 V-bottom plate.
13. After dispensing, balance keep back to the 15mL falcon. Add in MACS to wash -> Centrifuge and withdraw supernatant.
14. Add in total 750uL MACS to the pellet. (700+50uL extra)
15. Add in 100uL into each 7 Eppendorf. Add in antibody for staining.
16. Stain for 30 mins RT.
17. Add in 1mL MACS into each Eppendorf.
18. Invert the Eppendorf. Spin down at 5min 1500rpm. Withdraw supernatant
19. Add in 100uL of MACS into Eppendorf and resuspend.
20. Transfer the 100uL of the MACS in Eppendorf to 96 U-bottom plate. (JUST FOR DAY 0) (DAY 3 USE V-Bottom Plate)

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| **Patient 1** | **-ve** | **11b** | **33** | **34** | **38** | **DAPI** | **All** |
| **Patient 2** | **-ve** | **11b** | **33** | **34** | **38** | **DAPI** | **All** |

#Setting for machine. Use the primary sample template. Delete the well screen (all rows) BUT remain one last as template and rename the experiment. Save as different experiment before run.

Other information:

Limitation: weak positive CD34+ cells cannot be sorted out.

MS column: 1 mL wash, 2 mL elute, smaller magnet

LD column: depletion for CD34- (pure)

* Quiscent CD34-proliferation

Stem cell characteristic. Usually CD34+ around 1%, LMP, GMP, HSC

* If Normal control- Use PBSC

If Overexpression Experiment- Use Cord Blood