**Protocol for preparing primary AML samples for scRNA sequencing (based on 10X Genomics)**

Preparation

a) Warm a water bath to 37°C prior to commencing the thawing Protocol.

b) Prepare 35 ml warm complete growth medium (e.g. 10% FBS in IMDM) per sample by incubating in a 37°C incubator prior to use.

c) Prepare 1X PBS with 0.04% BSA (400 μg/ml) solution.

d) Prepare 1X Binding Buffer from the 20X Binding Buffer stock solution provided in the MACS® Dead Cell Removal Kit with Nuclease-Free Water

e) All steps use **wide-bore** pipette tip unless stated otherwise

**Dead Cell Removal & Washing**

1. After count cell-> Use strainer as a sieve. Pipette go through strainer into the 50mL Falcon. Pour through the 2% FBS in HBSS (~25mL). \* Wash the previous 50mL Falcon tube with 2%FBS in HBSS (~25ml)->Pour through the sieve.
2. Centrifuge cells at 1,200 rpm for 5 min.
3. Remove supernatant without disturbing pellet. Add 100 μl Dead Cell Removal MicroBeads (per 107 total cells) and resuspend pelleted cells using a wide-bore pipette tip.
4. Incubate for 15 min at room temperature (20 – 25°C). Prepare the 15mL Falcon tube. Add in 5ml 2% FBS in HBSS into the falcon tube.
5. (Pre wet) Rinse the MS column with **500 μl 1X Binding Buffer** while the cells are incubating with the Dead Cell Removal MicroBeads.
6. After incubation is complete, dilute the cell suspension (containing Dead Cell Removal MicroBeads) with **500 μl 1X Binding Buffer.**
7. Apply cell suspension to the prepared column. The positively selected dead cells will be retained on the column while the negatively selected live cells pass through the column. Eg CD34+ stick to the black.
8. **Wash the remaining in the Falcon (the balance cell) with 1mL Dead Cell Buffer through the column to wash.**
9. Collect the effluent containing the live cell fraction in a sterile 15 ml polypropylene carbon.centrifuge tube.
10. Rinse the column with 2 ml 1X Binding Buffer and combine with the original effluent.
11. Centrifuge cells at 1,200 rpm for 5 min.
12. Remove the supernatant without disturbing the pellet.
13. Using a wide-bore pipette tip, add 1 ml 1X PBS containing 0.04% BSA to each tube and gently pipette mix 5 times to resuspend cell pellet. Transfer the cell suspension to a 2 ml tube.
14. Centrifuge cells at 1,200 rpm for 5 min.
15. ~~Repeat 17-18.~~
16. ~~Count cells. Resuspend in final 2 x 10~~~~6~~ ~~cells per mL. Use ~200 μl for sample submission and the remaining for cytospin (200 μl per slides), FACS analysis, DNA, RNA.~~

**FITC -11b (Differentiation)**

**APC -CD34+ (Proliferation)**

**Pacific Blue- CD38**

**PE – CD33**