CD56-Antibody Staining of Primary Human Myoblasts For FACS Analysis:

You will need:

- Phosphate Buffered Saline (PBS; Cellgro, 45000-448)
- TrypLE Express (Gibco, 12605-028)
- APC Mouse Anti-Human CD56 (BD Biosciences, 555518)
- APC-Mouse IgG1 K isotype Control (BD Biosciences, 555751)
- 10 % FBS-PBS solution (store at 4°C):
  1mL Fetal Bovine Serum (FBS, characterized; Hyclone, SH3007103)
  9mL PBS (Cellgro, 45000-448)
- 15mL centrifuge tubes
- 5mL polystyrene round-bottom FACS tubes (BD Falcon, 352058)
- 6cm and 10cm tissue culture dishes coated with 0.1% gelatin (Sigma, G9391-100G)
- ~ $1x10^6$ primary myoblasts
- Growth medium:
  20% FBS, characterized (Hyclone, SH3007103)
  0.5% chick embryo extract
  1.2mM $CaCl_2$ (EMD, 10035-04-8)
  1% antibiotic/antimycotic (Cellgro, 45000-616)
  in Ham's F-10 (Cellgro, 45000-356)
- 2x freeze medium:
  50% FBS, characterized (Hyclone, SH3007103)
  20% DMSO (Alfa Aesar, AA43998-5Y)
  30% growth medium

Procedure:

1. Culture human primary myoblasts on 10cm dishes coated with 0.1% gelatin, feeding daily, until cells reach 50-70% confluence.
2. Trypsinize cells: aspirate medium and rinse cells with 10mL of PBS/dish; add 2mL of TrypLE and incubate at 37°C for 3-5mins until all cells have detached.
3. Neutralize TrypLE with an equal volume of growth medium and disperse cells with a serological pipette until a single cell density is reached. Transfer cells to a 15mL tube.
4. Vortex gently, and count 10µL on a hemocytometer.
5. Centrifuge cells at 1000 rpm for 5 mins.
6. Resuspend cell pellet in 500µL PBS and re-spin; aspirate liquid and repeat wash 2 times.
7. Resuspend pellet in 10% FBS + PBS (110µL for ~$1x10^6$ cells)
8. Prepare two FACS tubes for each sample: label one with sample ID and CD56. Label the other tube with sample ID and Isotype Control; add 90µL of 10% FBS-PBS to this tube.
9. Split the sample so that 100µL is added to the tube labeled CD56 and 10µL is added to the isotype control tube. (Each tube will have a total volume of 100µL.)
10. Add 1° antibody
    a. CD56 – add 20µL of APC-CD56 antibody
    b. Isotype Control – add 20µL of APC-mouse IgG1 isotype control

11. Incubate for 30-60 mins. on ice in the dark.
12. Centrifuge at 1000 rpm for 5mins.
13. Resuspend cells in 500μL PBS and re-spin, repeat wash 1 time.
14. Resuspend pellets as follows: 500μL of 10% FBS-PBS for the isotype control and 1mL of 10% FBS-PBS for the CD56 sample
15. Run samples on FACS Aria (BD Biosciences) or equivalent cell sorter – for each sample record the following data:
    a. Isotype Control: collect at least 1000 events to identify the negative population
    b. Pre-sort: collect at least 1000 events to gauge the percentage of CD56+ cells in the starting population
    c. Sort: collect at least 1000 events to record the populations collected
    d. Post-sort CD56+ : collect between 500-1000 events depending on percents to verify positive population
    e. Post-sort CD56- : collect between 500-1000 events depending on percents to verify negative population
17. Plate the CD56+ population and the CD56- population on their respective plates for expansion in growth medium (seed cells at density of ~2000-3000 cells/cm2); feed daily and expand until cells reach 50-70% confluence. To freeze cells: trypsinize, neutralize, and count cells as above, adjust volume as appropriate with growth medium, and add an equal volume of 2X freeze medium and mix well. Aliquot into cryovials and freeze in liquid nitrogen vapor phase.