STAINING PROCEDURE USING ALL DIRECTLY CONJUGATED REAGENTS

1. Prepare lysing reagent :
   4.13 g Ammonium Chloride (NH_4Cl) -- Sigma A-5666
   0.5 g Potassium Bicarbonate ($KHCO_3$) -- Sigma P-4913
   0.0185 g Tetra Sodium EDTA -- Sigma ED4SS

(All above chemicals must be less than one year old and stored tightly sealed.) Measure carefully the above chemicals. Pour into a flask (must be at least a 500 ml flask) to which a stir bar has been added. Add 500 ml double distilled water. Place on stir plate and stir until the powder is dissolved.
Do not pH. Scale quantities for daily use: must be made fresh daily. Use at room temperature or warm to 27°C.
PREPARE FRESH SOLUTION DAILY!

We recommend weighing the reagents and storing them as packets. The dry reagents are dissolved in water as required.

2. Label 12 x 75 mm plastic Falcon #2054 tubes with donor name, date, antibody panel, (including standards); put all tubes on ice.

3. Dispense all antibody combinations into appropriately labeled tubes.

4. Put 2.0-3.0 ml whole blood into a 15ml centrifuge tube and q. s. to 15 ml with PBS containing heparin (10 u/ml or 1 ml/ litre).

5. Centrifuge @ 400 x g (1400 rpm*) for 3 min., aspirate supernatant carefully. Mix cell suspension well. Repeat wash with PBS. Resuspend in PBS, final volume 2.0 mL

6. To block cell suspension add 200 µg of normal mouse IgG (Caltag 10400) at 1 mg/ml (or 67 µl of 3 mg/ml) for each ml of the washed cell suspension and incubate for 10 minutes on ice, then add 80 µL of this suspension to each of the appropriate tubes. **Rack each tube after adding. Incubate another 15 min. on ice.

7. Add 3.5 ml lysing reagent to each tube, put a cap on each tube (or use parafilm over all tubes), invert once and place directly into a centrifuge adaptor, incubate for 5 minutes. Do not lyse more than 42 tubes at a time. Invert all once or twice and centrifuge @400 x g (1400 rpm*) for 5 min. Remove caps (or parafilm), decant the supernatant and blot on teri towel three times. Rack tubes and replace in adaptor.

8. Add 3.5 ml PBS, centrifuge as above, decant, blot three times and resuspend pellets in residual PBS. Note: do not leave samples in this state, fix immediately on ice.

9. Fix all samples in 0.5 ml of 2% formaldehyde. (Polysciences, cat. # 04018 Ultrapure, E. M. Grade 10% solution. Dilute to 2% in PBS.) Samples may be run after 30 minutes, however, it is best to wait 6 hrs. for the cells to stabilize.

** note: for blocking only 50 µl of washed blood : use 10 µl of mIgG @ 1 mg/ml and add antibodies after 10 min. of incubation. Do not wash between adding mIg and antibodies.

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