MONOCLONAL ANTIBODY STAINING PROCEDURE FOR WHOLE BLOOD USING NH_4CL FOR LYSIS OF RED BLOOD CELLS

I. SAMPLE

A) Blood drawn into EDTA vacutainer tubes, keep at room temperature and process within 24 hours.

B) Blood drawn into Heparin vacutainer tubes, keep at room temperature and process within 24 hours.

II. REAGENTS

A) Antibodies

1. Primary antibodies: purchased or your own

a) If these are conjugated with a fluorochrome (i.e., FITC, PE, or other) use the DIRECT STAINING PROCEDURE.

b) If the primary antibody is not fluorochrome-conjugated, use the INDIRECT STAINING PROCEDURE.

2. Secondary antibodies: fluorochrome-conjugated polyclonal antibody

B) Buffer: PBS (e.g., Ca^{2+} and Mg^{2+} free, Irvine Scientific, cat. #9240) + 2% newborn calf serum (or 0.2% BSA) + 0.1% sodium azide.

C) NH4Cl Lysing Solution (see attached recipe and commercial source)

III. PROCEDURE

DIRECT STAINING

1. First, add 50 µL of BUFFER, then the appropriate amount of monoclonal antibody to the bottom of tubes.
Note: For dual- or multi-color staining, add all your directly-conjugated antibodies at the same time.

2. Add 50 µL of whole blood to the bottom of the tubes.

3. Vortex briefly and incubate 15 minutes at room temperature.

4. Wash twice with ~1mL of buffer; centrifuge at 250g for 5 minutes.

5. Take off as much buffer as possible without disturbing the pellet.

6. Vortex briefly and add 1 ml room temperature 1 x NH4 CL lysing solution to the pellet.

7. Vortex immediately and incubate samples between 7 and 10 minutes at room temperature protected from light.
Note: Do not exceed this time.

8. Spin down samples for 5 minutes at 200 g.

9. Take off lysing solution as completely as possible.

10. Resuspend samples in ~ 0.5 mL of buffer and hold in refrigerator (or on ice) protected from light prior to analysis.

INDIRECT STAINING

1-4. Process cell samples as above using a working dilution of unlabeled monoclonal antibody.

5. Resuspend cell pellet in 100 µL of working dilution of the fluorochrome-conjugated second antibody.

6. Vortex briefly and incubate for 20 minutes at room temperature.

7. Wash twice with ~1mL of buffer; centrifuge at 250g for 5 minutes.

8. Process samples as described above in 5-10 of DIRECT STAINING procedure.

Note: If you increase the amount of whole blood you stain you have to increase the amount of lysing buffer to maintain a ratio of 1:20 of blood to lysing solution.

AMMONIUM CHLORIDE LYSING SOLUTION – 10X

Reagents	Amount:
Ammonium Chloride, ACS	82.9 g
Potassium Bicarbonate, USP	10.0 g
Ethylenediamine tetraacetic acid (EDTA) disodium salt	0.37 g
Water, glass-distilled	qsad 1.0 liter

Adjust pH to 7.2 and keep in tightly closed container at 4°C. To prepare a 1X working solution (to be used at room temperature), dilute 10X 1:10 with glass distilled water. Keep tightly closed and discard at the end of the day. **Note** that 10X NH4CL Lysing solution can be obtained commercially, e.g. from BD Pharmingen, CA, PharM Lyse™ Cat# 555899.