BLOCKING OF FC-RECEPTOR BINDING WITH HUMAN AB SERUM (HAB)

If your cells have many Fc-receptors on the cell surface (in particular monocytes, macrophages) or they have been cultured in serum-free medium, it is advisable to block nonspecific binding of monoclonal antibodies by pre-incubation of cells with human AB serum (HAB). Note that for staining of whole blood this is not necessary, because serum in high concentration is present during staining.

I. REAGENTS:

A. Antibodies

1. Primary antibodies: usually purchased or your own monoclonals

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 conjugated to a fluorochrome, use the INDIRECT STAINING procedure.

2. Secondary antibodies: fluorochrome-conjugated polyclonal antibody

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

C. HAB (e.g., from Innovative Research, MI), either purchase heat-inactivated HAB or inactivate it by heating it to 56^{o}C for 1h. Divide into small aliquots and keep frozen at -20°C.

II. PROCEDURE:

DIRECT STAINING

1. Prepare your cells as a suspension of single cells in a manner appropriate for the specimen you wish to examine. As the final step, wash at least once with 1 ml of cold BUFFER. Re-suspend the cells at 10^7 cells/ml (thus 50 µL= 5 x 10^5 cells) in cold BUFFER.

Check cell viability, it should exceed 90%. If cell viability is less than 90%, remove dead cells by Ficoll-Hypaque separation, otherwise dead cells will bind antibodies nonspecifically. Preferably, add a dead cell discriminating dye for exclusion of dead cells from flow cytometric analysis (see protocols for adding either propidium iodide or 7-amino-actinomycin D to the cells in the final re-suspension step before acquisition on the flow cytometer or follow procedures as outlined by manufacturers of other dead cell discrimination dyes).

2. Add 50 µL of the cell suspension to the bottom of the tubes.

3. Add 50 µL of HAB to each tube mix well and incubate for ~1 min at room temperature.

4. Then, add the appropriate amount of monoclonal antibody to the bottom of tubes sitting on ice.

Note: For multi-color staining, add all your fluorochrome-conjugated antibodies at the same time.

5. Vortex briefly and incubate for 30 min at 4°C in the dark.

6. Wash twice with 1mL of buffer and hold them at 4°C (or on ice) prior to acquisition on the flow cytometer.

INDIRECT STAINING

1-6. Process cell samples as above using a working dilution of unlabelled monoclonal antibody.

7. Resuspend cell pellet in 50 µL HAB, mix and incubate for ~1 min at room temperature.

8. Add 50 µL of working dilution of the fluorochrome-conjugated second antibody.

9. Vortex briefly and incubate for 20 min at 4°C in the dark.

10. Wash twice with 1 mL of buffer; centrifuge at 250g for 5 min.

11. Resuspend samples in 1 mL of buffer and hold them at 4°C (or on ice) protected from light prior to acquisition on the flow cytometer.

Note*:* This method cannot be used for staining of surface Ig or staining with antibodies that are directed against Fc-receptors (e.g., CD16).