Extracellular Staining Protocol

1. Add 0.5 x 10^6 - 1 x 10^6 cells to 1.5 ml eppendorf tube. Check cell viability (i.e. trypan blue exclusion), 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.

2. Spin down cells at 1200-1800 rcf (3600-4400 rpm) for 5 minutes at 4°C (use microcentrifuge).

3. Remove supernatant.

4. Resuspend cell pellet in 50 µl of staining buffer# (1X PBS /1% FCS*, 1X PBS/1% CS**, or 1X PBS/1% BSA&) containing <1 µg of FcR block Ab (e.g., FcBlock clone 2.4G2) and incubate 5 min/4°C.

5. Without washing, add 50 µL of the appropriate concentration of conjugated monoclonal antibodies (use optimal Ab concentration determined by titration for optimal staining: 10, 5, 2.5, 1.25, etc. µg/mL. Please, refer to Antibody Titration protocol). If using a biotin conjugate, always stain with this antibody first and by itself (DO NOT USE BIOTIN FOR INTRACELLULAR STAINING).

6. Incubate on ice 30 minutes (foil covered).

7. Wash 2x with 1.0 ml of staining buffer.

8. Spin down cells at 1200-1800 rcf (3600-4400 rpm) for 5 minutes at 4°C (use microcentrifuge).

9. If using a biotin conjugate, resuspend cell pellet in 50µl of staining buffer (1X PBS /1% FCS) containing streptavidin conjugated dye plus other directly conjugated antibodies, repeat steps 6 through 8.

10. Resuspend cell pellet in 300 µl of staining buffer (better to analyze unfixed cells on the same day).

11. An unstained sample (or Ig isotype control) and single stained samples for each conjugated dye are also necessary for multicolor experiments (Compensation Controls).

12. Cells can be fixed using 1-4 % of paraformaldehyde/PBS-CS for about 30-60 min/4°C.

13. Wash 2x with 1.3 ml of staining buffer.

14. Spin down cells at 1200-1800 rcf (3600-4400 rpm) for 5 minutes at 4°C (use microcentrifuge).

15. Resuspend cell pellet in 300µl of staining buffer. Fixed cells can be run later (depend of the fluorochrome, samples can be run 1 week after staining).

#It can be add 0. 1% (w/v) sodium azide
*Fetal Bovine Serum
**Calf Serum
&Bovine Serum Albumin