**Live Cell Surface Staining**

* Adjust cell concentration to 60x106 cells/ml in Maxpar Cell Staining Buffer (CSB, Fluidigm) including Fc block (eBioscience Cat # 14-9161-73 or similar product).
* Incubate cells with Fc block at room temperature for a minimum of 10-min before adding cell suspension to antibody cocktail.
* Add 50 μl cells (3 x 106 cells) to 50 μl antibody cocktail. Total staining volume is 100 μl (50 μl of cell suspension + 50 μl antibody cocktail).
* Gently mix samples and incubate for 30 minutes at room temperature.
* Following the incubation, wash by adding 2 ml CSB to each tube. Centrifuge (300xg) and discard supernatant by aspiration.
* Repeat for a total of two washes, and resuspend cells in residual volume by gently mixing after final wash/aspiration.
* Hard fix cells by incubating in 1 ml 2% paraformaldehyde in PBS (Rockland, Cat#MB008 diluted in 18 MOhm water) for 1-hr at 4°C. Make sure to use fresh EM-grade paraformaldehyde reagent for this step (usually provided at 16% in glass ampules). [If also staining for intracellular antigens, conduct the hard fix after intracellular staining.]
* All centrifugations after fixing cells should be at 800xg.
* Follow with mass cytometry intracellular stain (optional) or Iridium stain protocol.