FLOWMETRIC CASE STUDY
CELL SIGNALING ASSAYS

FlowMetric™

Objective:
In order to better assess cell functionality, FlowMetric was tasked to analyze key phosphoproteins in lymphocyte subsets without prior isolation from whole blood patient donor samples.

Method:
- Fresh whole blood from normal healthy human donors was stimulated with either hIL-6 or hIL-2 at a concentration of 100ng/mL for 15 minutes at 37°C.
- Cells were processed using a phosphoflow assay. Cells were lysed and fixed, washed and permeabilized for 30 minutes at 4°C.
- Cells were then stained for surface markers for CD3, CD4, CD8 and CD19 for 1 hour at room temperature.
- Cells were then washed, resuspended in 500µL Stain Buffer and acquired using a BD Fortessa™.

Results:
The following examples show activated CD4+ and CD8+ T cell signaling profiles for each of the phosphoprotein markers, STAT-3 and STAT-5. The CD19+ B-cells were negative for both of these markers.

Nested Gating Strategy

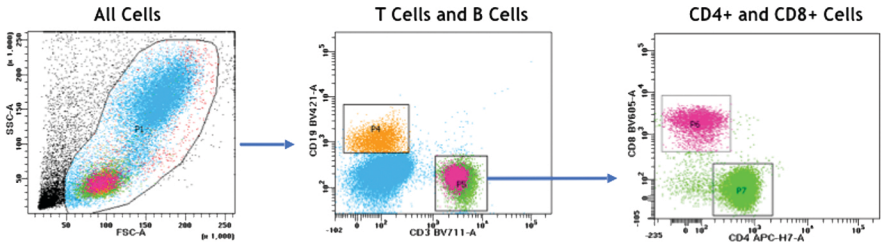

Figure 1. The first plot shows a light scatter gate used to identify peripheral blood cell populations. Next, a CD3+ and CD19+ population is identified. Finally, CD8+ and CD4+ T Cell populations are identified within the CD3+ population.

Detection of Heterogeneous Signaling Responses Using PhosphoFlow

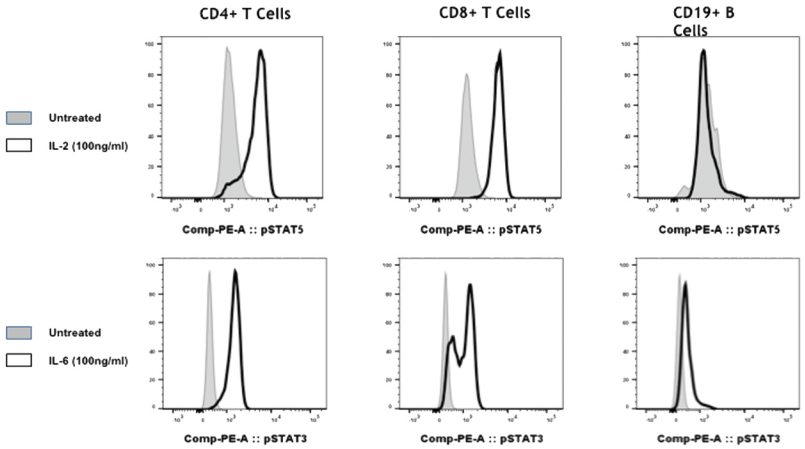

Figure 2. Activation profiles for CD4+ and CD8+ T cells as well as CD19+ B cells. The data are displayed as histogram overlays with untreated cells. These histograms show signaling responses of each phosphorylation marker, STAT-3 and STAT-5, induced by the corresponding response modifier, hIL-6 (100ng/mL) for STAT-3 and hIL-2 (100ng/mL) for STAT-5.