

Nolan Laboratory General Phospho-Flow Background

Phosphorylation of signaling proteins within cells modulates their activity. In many cases, phosphorylation leads to the activation of the protein (MAP kinases, Stat transcription factors), while in some cases, phosphorylation maintains proteins in an inactive state (Lck, for example). Therefore, measurement of phosphorylation of specific residues on these signaling proteins is critical to understanding how signaling cascades work. Recently, phospho-specific antibodies have been developed that *only* recognize a specific phosphorylated residue on a target protein. These antibodies do not cross react with the non-phosphorylated form of the epitope. This way, the “on/off” state of a signaling protein can be measured.

Though many of these antibodies have been used and validated by Western blot, it has only been in the last few years that researchers have begun to apply them to flow cytometry. Among the molecules examined to date are Stat1, Stat4, Akt, cJun and p38, MEK and Erk, and others. Flow cytometry has the unique ability to quantify these phosphorylation events within individual cells, whereas Western blotting averages thousands of cells to get one data point. In addition, flow cytometry can correlate multiple epitopes simultaneously, whether phospho-epitopes or surface markers.