

## **Simultaneous Detection of Key Signaling Proteins in T-Cell Activation Pathways Using Multiplex Suspension Arrays**

**Paul Wilson Rhyne**<sup>1</sup>, Imran H. Khan<sup>2</sup>, Sara Mendoza<sup>2</sup>, Joe Tuscano<sup>2</sup>, Hsing-Jien Kung<sup>2</sup>, Dominic Eisinger<sup>1</sup>, and Paul A. Luciw<sup>2</sup>: <sup>1</sup>Upstate USA, 10 Barn Rd, Lake Placid, NY 12962, <sup>2</sup>UC Davis, Ctr. For Comparative Medicine, Davis CA 95616.

### **Abstract**

We describe the use of multiplex suspension arrays to study changes in phosphorylation of signaling proteins in T-cell activation pathway. The array consists of color-coded microbead sets covalently coupled to capture antibodies specific to each protein target. A second biotin labeled reporter antibody recognized the phosphorylation site on the protein target, followed by the addition of streptavidin-PE. Phosphorylation of each protein was detected from a single 10  $\mu$ l sample of cell lysate using the Luminex<sup>100</sup> multiplex system. We show that the array detected phosphorylation changes in the TcR/CD3 receptor, Lck, Zap70, LAT, PKC alpha, Akt, CREB, Erk, Rsk, and STAT3 in both anti-CD3 and sodium pervanadate treated cells. These changes were not due to increased protein expression levels after treatment as determined by a suspension array that detects these proteins irrespective of phosphorylation. These results were confirmed by Western analysis. B cell receptor associated kinase, Syk, was not affected by the anti-CD3 treatment. Thus, the bead array provides an efficient method for simultaneous study of several key signaling events. This method may facilitate molecular profiling of signaling proteins associated with a variety of diseases, e.g., cancer.