

**P014** Competitive Fluorescence Polarization Assays for the Detection of Phosphoinositide Kinase and Phosphatase Activity

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We describe implementation of competitive fluorescence polarization (FP) based assays for determining activity of phosphoinositide 3-kinase (PI 3-K) and the type-II SH2-domain-containing inositol 5-phosphatase (SHIP2). Assays are based on the interaction of phosphoinositide binding proteins with fluorophore-labeled phosphoinositide and inositol phosphate tracers. Enzyme reaction products are detected by their ability to compete with fluorescent tracers for protein binding, leading to an increase in amounts of free tracer and a decrease in polarization (mP) values. A variety of fluorophore-labeled tracers were evaluated. Assay sensitivity and specificity for products of PI 3-K and SHIP2 activity was determined, and performance was evaluated using recombinant PI 3-K $\alpha$  and SHIP2 with diC<sub>8</sub>-PI(4,5)P<sub>2</sub> and diC<sub>8</sub>-PI(3,4,5)P<sub>3</sub> as respective substrates. IC<sub>50</sub> values for previously characterized PI 3-K inhibitors were within expected ranges. These assays are homogeneous, sensitive, rapid, and suitable for HTS applications, and will facilitate screening for novel inhibitors of phosphoinositide kinases and phosphatases in drug development.