

substrate. Phosphorylation of the substrate by the kinase produces a substrate that binds to the donor beads through the streptavidin/biotin interaction and to the acceptor bead via an antibody/antigen interaction. This positions the donor and acceptor beads in close proximity and singlet oxygen produced upon irradiation at 680 nm produces a fluorescent response in the acceptor beads. Compounds that interfere with kinase activity will block phosphorylation of the substrate, preventing the formation of bidentate interaction that brings the donor and acceptor beads together, thus decreasing the intensity of the fluorescent signal. Quantification of the changes in signal intensity produced in the presence of various concentrations of potential kinase inhibitors allows IC_{50} determinations for test compounds (Figure 4.25).⁵⁶

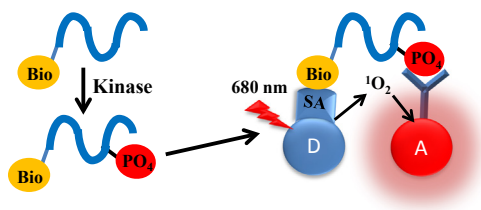


FIGURE 4.25 In the presence of an appropriate kinase, a biotinylated peptide is phosphorylated. When streptavidin-labeled donor beads and acceptor beads tagged with an antibody to the phosphorylated peptide are added, the donor/acceptor pair is brought together. Irradiation with light induces a fluorescent response in the acceptor bead. Compounds that block kinase activity will decrease fluorescent intensity in a concentration-dependent manner.

AlphaScreen technology has found widespread acceptance in the pharmaceutical industry. Applications for this assay system have been developed to monitor enzyme activity,⁵⁷ identify compounds that are functionally active in signal transduction cascades,⁵⁸ and to study the formation and disruption of macromolecular complexes.⁵⁹ As with FRET and TRFRET technology, as long as one can design a system in which a biological event mediates the relative position of the donor and acceptor beads, an AlphaScreen assay is possible.

Fluorescent Detection of Calcium Flux

The ability to detect changes in cellular calcium utilization and mobilization has led to the development of numerous assay systems designed to generate fluorescent signals based on calcium concentration. Some of these systems depend on calcium-sensitive dyes such as Fluo-3 and Fluo-4