longer than typical background fluorescent sources. Background emission sources such as cellular material, plastics, and small organic compounds typically fluoresce in time windows measured in microseconds, while lanthanide-based organometallic systems fluoresce with decay rates that extend into millisecond time scales. Thus, a small delay between initial excitation and fluorescent detection, 50–150 μs, creates a measurement window during which background fluorescence has occurred and extinguished itself, but the lanthanide acceptor is still fluorescent, eliminating a significant amount of background fluorescence (Figure 4.20). Commercially

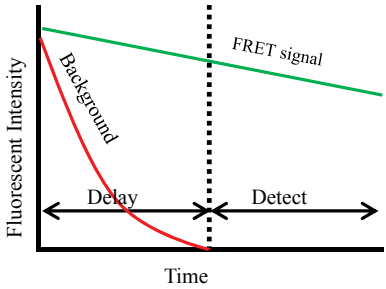

FIGURE 4.20 Irradiation of the components of a TRFRET assays system produce both background fluorescence and the FRET signal associated with the donor/acceptor pair. The application of lanthanide-based organofluorophores, however, extends the duration of the FRET signal beyond the decay limit of background fluorescence. This provides a time window for measurement of FRET signal with improved signal to noise ratios.

available TRFRET systems include Perkin Elmer's Lance® technology that utilizes europium chelates,[50] Cisbio's HTRF® assay systems that employ europium and terbium cryptates,[51] and Invitrogen's LanthaScreen® platforms that take advantage of terbium and europium chelates.[52]

Much like FRET technology, as long as it is possible to label a biological system with a donor/acceptor pair that will associate or disassociate as a result of a biological process, it is possible to apply TRFRET technology to quantify the biological activity of test compounds. Kinase activity, for example, can be measured through the use of antibodies tagged with a TRFRET fluorophore (Figure 4.21). In this case, a biotinylated kinase substrate could undergo phosphorylation, and the resulting phosphorylated species would

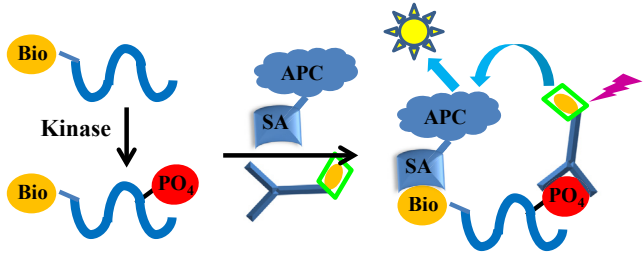

FIGURE 4.21 In this TRFRET kinase assay, phosphorylation of the biotinylated (Bio) target by a kinase produces an antigen capable of interacting with an antibody tagged with a lanthanide-based fluorophore. Addition of a streptavidin (SA)-linked fluorescent protein such as Allophycocyanin (APC) establishes a donor/acceptor pair that will produce light via a FRET pathway upon irradiation.