Within the context of reporter assays, the application of luciferase technology is essentially the same as CAT and β-lactamase reporter assays systems. Coupling of the expression of a target gene with a luciferase gene creates a situation in which compounds that promote the expression of the target gene also promote the expression of luciferase. In the presence of the proper substrate, increased gene expression leads to increased light emission that can be quantified as means of monitoring gene transcription. Conversely, compounds that block expression of the target gene will also decrease the production of luciferase, providing a means of identifying compounds that prevent gene expression.[67]

Both the β-lactamase and luciferase reporter assays systems avoid the use of radiolabeled materials, but luciferase reporter assay systems have an additional advantage. As discussed earlier, β-lactamase reporter systems depend upon the presence or absence of a FRET signal, which requires light irradiation at the acceptor compound's absorbance frequency and detection of the donor compound's emission frequency. Potential fluorescent interference from the assay media, cellular material, assay plates, etc. is an issue in FRET-based reporter assay in the same manner as it is in any other type of FRET-based assay. Thus compounds that fluoresce or act as fluorescence quenchers can produce misleading results in FRET reporter systems.

Luciferase reporter assays, on the other hand, generate light emission as a consequence of the chemical reaction mediate by the enzyme and do not require irradiation with an external light source. As such, the risk of interference via fluorescence of other assay components (assay media, cellular material, assay plates, etc.) is significantly lower. Of course, compounds that inhibit luciferase activity will impact screening results. Counterscreening of compounds for luciferase inhibition in the absence of the reporter gene system is an important aspect of data analysis in luciferase reporter gene assay.

KINETIC FLUORESCENT MEASUREMENT SYSTEMS

While there are many biological processes that can be measured with simple fluorescent plate readers, there are some cellular events that are transient in nature. Changes in membrane potential, calcium mobilization events, and GPCR activation can occur on an extremely short timescale and are often transient in nature. Membrane potential in cardiomyocytes, also shifts rapidly and in opposing directions with each heartbeat. Monitoring the kinetic time courses of these events can provide a wealth of information on the impact of test compounds, provided it is possible to measure changes in signal emission within the event window. The Fluorescent Imaging Plate Reader (FLIPR) developed by Molecular Devices[79] and the Functional Drug Screening System (FDSS) [80] produced by Hamamatsu Photonics were both designed to address this need. Although there are some differences