requires the use of radioactive material, and as such, necessitates isolated equipment, staff skilled and certified in the use of radiolabeled material, as well as the generation and disposal of radioactive waste material. These drawbacks must be considered before committing to the prosecution of an assay incorporating radiolabeled material, conventional or SPA, as other technologies may be able to provide similar data output at a lower cost.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The binding interaction between an antibody and its target antigen is both highly stable and exceptionally selective. These features have led to the development of a number of assay systems designed to take advantage of the highly specific nature and strength of the antigen/antibody complex. Radioimmunoassay designed to monitor biological processes, particularly the concentration of antigens in blood samples, were described in by Rosalyn Yalow and Solomon Berson as part of their efforts to quantify blood insulin levels.[20] Their methods, along with many modernized variations, are still employed today, as radioimmunoassays are both extremely sensitive and inexpensive. Efforts to move away from the use of radioactive material, however, led to the development of a similar assay system that eliminates the need for radiolabeled material.

The enzyme-linked immunosorbent assay, more commonly referred to as ELISA, takes advantages of the positive aspects of radioimmunoassay techniques, but quantification of biological signals is accomplished by monitoring enzymatic activity instead of changes in radioactivity. Originally developed independently by Anton Schuurs and Bauke van Weemen in the Netherlands[21] and Peter Perlmann and Eva Engvall at Stockholm University in Sweden,[22] ELISA methods employ antibodies that have been linked to an enzyme capable of generating a signal when in the presence of a specific substrate. In many cases, the signal change is a colorimetric change that can be observed with a simple spectrophotometer. In the direct ELISA method, the surface of a microtiter plate is coated with an antigen and an enzyme-linked antibody is added. Removal of unbound antibodies using standard plate washing methods is followed by addition of a substrate that can be converted to a colored material by the enzyme. Monitoring the rate of appearance of color can be used to quantitate biological material or biological events. If the amount of antigen applied to the plate is *unknown*, then the rate of color change will be directly related to the amount of antigen applied to each well, and the amount of antigen in solution can be determined based on colorimetric changes. Alternatively, if the amount of antigen applied to each well is a *known constant*, then the rate of color change can be used to identify enzyme inhibitors. The rate of color change will decrease in the presence of compounds that block enzyme activity (Figure 4.9).