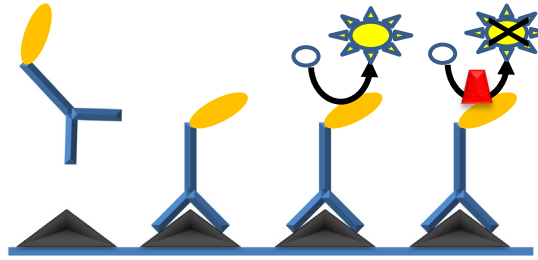

FIGURE 4.9 A surface coated with an antigen (black) is treated with a compatible antibody that has been covalently linked to an enzyme (orange). Addition of a suitable substrate (open blue circle) will produce a color change (yellow) whose intensity is dependent upon the amount of substrate and antigen present. The presence of an enzyme inhibitor (red) can also be detected based on changes the intensity of color produced.

Another important variation of the ELISA technique, the sandwich ELISA, requires multiple antibodies, the last of which is linked to an enzyme that can be used to provide a quantitative signal. In one scenario, a microtiter plate (or other surface) is coated with a capture antibody. Application of a solution containing an antigen that interacts with the antibody forms a stable complex, and standard plate washing techniques are employed to remove any unbound material. The addition of a second antibody linked to an enzyme capable of producing a signal and binding to the antigen produces a three-membered complex that sandwiches an antigen between two antibodies. Addition of an appropriate enzyme substrate produces a signal that can be monitored and quantified (Figure 4.10). While sandwich ELISAs are employed in drug discovery, this technique has enjoyed broad

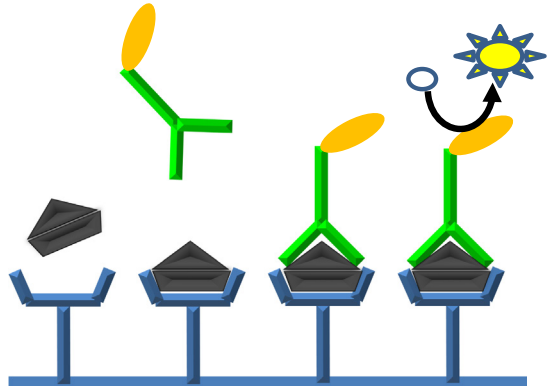

FIGURE 4.10 A surface coated with an antibody (blue) is treated with a solution containing a compatible antigen (black). The stable complex that is formed is then treated with a second antibody (green) linked to an enzyme (orange), creating a "sandwich" complex. Addition of a suitable substrate (open blue circle) will produce a color change (yellow) whose intensity is dependent upon the amount of substrate and antigen present.