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Direct ELISA

The direct detection method uses a labeled primary antibody that reacts directly with the antigen. Direct detection can be performed with antigen that is directly immobilized on the assay plate (Figure 1) or by first attaching a capture antibody to the plate surface (Sandwich ELISA, Figure 2). At last, the substrate will be added to complete colorimetric reaction by HRP-conjugated-antibody.

Figure 1

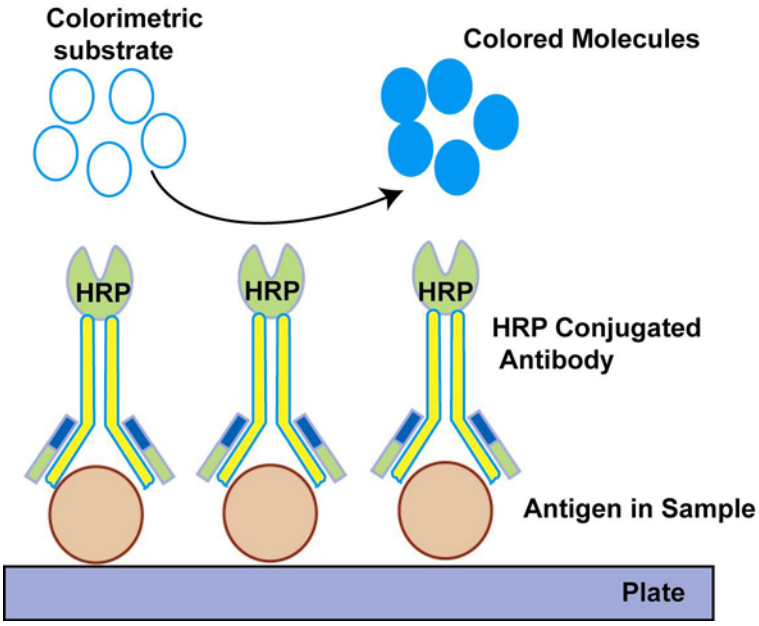

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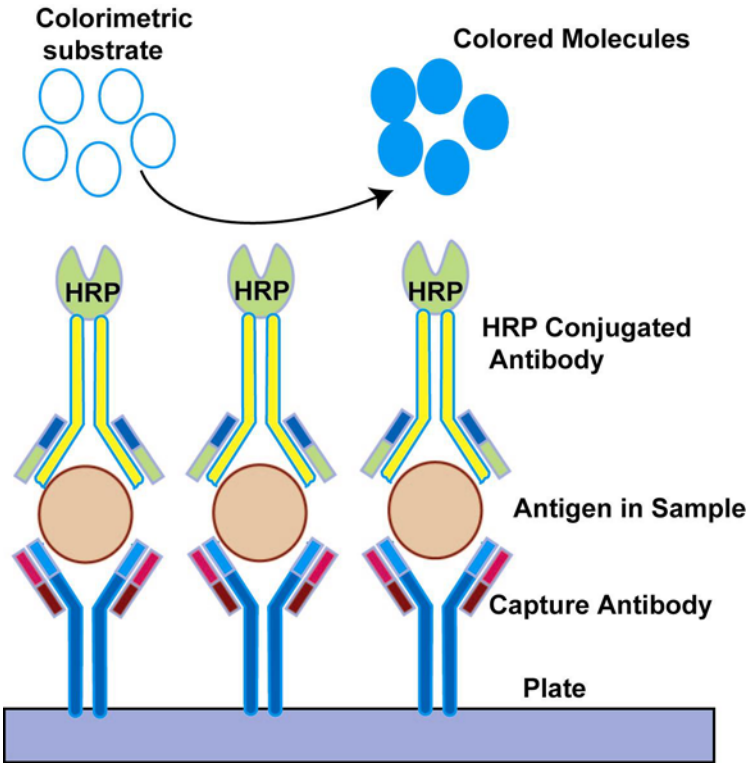
Figure 2



Competitive ELISAs

Competitive ELISA can be used to determine if a molecule is present in the sample or not, or to assess the concentration of antigen, hormone or small molecules including cAMP, cGMP, NO etc. First, the wells will be coated with secondary antibody. The primary antibody will be added to bind it. Then, molecules of interest (antigen) in sample will be added along with the HRP-conjugated-antigen. At last, the substrate will be added to initiate colorimetric reaction by HRP-conjugated-antigen (Figure3). The more intense the color is, the less molecules of interest is present. This is because the free antigen molecule binds the primary antibody and competes away the HRP-conjugated-antigen. If there is not much of the molecule of interest, the HRP-conjugated-antigen will bind to the primary antibody, therefore, stay with the plate to enhance the color.

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Figure 3