Enzyme-Immunoassay (EIA)

The colorimetric assay is based upon a competition between the unlabeled analyte (either varying concentrations of the standard or the test samples) and the biotinylated analyte for limited binding sites available on the primary analyte-specific antibody. The immunoplate is pre-coated with the secondary antibody. The primary antibody, biotinylated peptide, and non-biotinylated peptide (either standard or test sample) are mixed in a well. The primary antibody is captured by the immobilized second antibody. Unbound analytes are removed through a series of washes. A subsequent addition of streptavidin-horseradish peroxidase (SA-HRP), which binds to the biotinylated peptide, followed by the addition of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate turns the solution blue. Once the desired color intensity is achieved, the enzyme-substrate reaction is terminated by hydrochloric acid which turns the solution yellow. Thus, the absorbance reflects the amount of biotinylated peptide-antibody complex bound to the well. The concentration of the sample analytes can then be determined from the generated standard curve.