



Plasma Required: 25  $\mu$ l  
 Range of Values observed: 10 – 350 nM

Plasma 25-hydroxy vitamin D is measured utilizing an Immunoassay kit purchased from Immunodiagnostic Systems, Ltd., Fountain Hills, AZ. The assay employs an immunoassay technique utilizing a sheep antibody specific for 25-OH vitamin D precoated into wells of a 96 well microplate. Standards and samples are diluted with biotin labeled 25-OH vitamin D and incubated in the antibody-coated wells for two hours and then aspirated. After washing to remove any unbound substances, horseradish peroxidase labeled avidin is then added which binds selectively to complexed biotin. After removal of unbound conjugate a substrate solution is added to each well and color allowed to develop inversely proportionate to the amount of vitamin D present in the original sample. The color development is stopped with the addition of 1 M HCl and the microplate then read at 450 nm and also at 590 nm as a background control. Plots of concentration vs absorbance for standards are prepared using a four parameter fit and concentrations of unknown samples extrapolated from the standard curve.