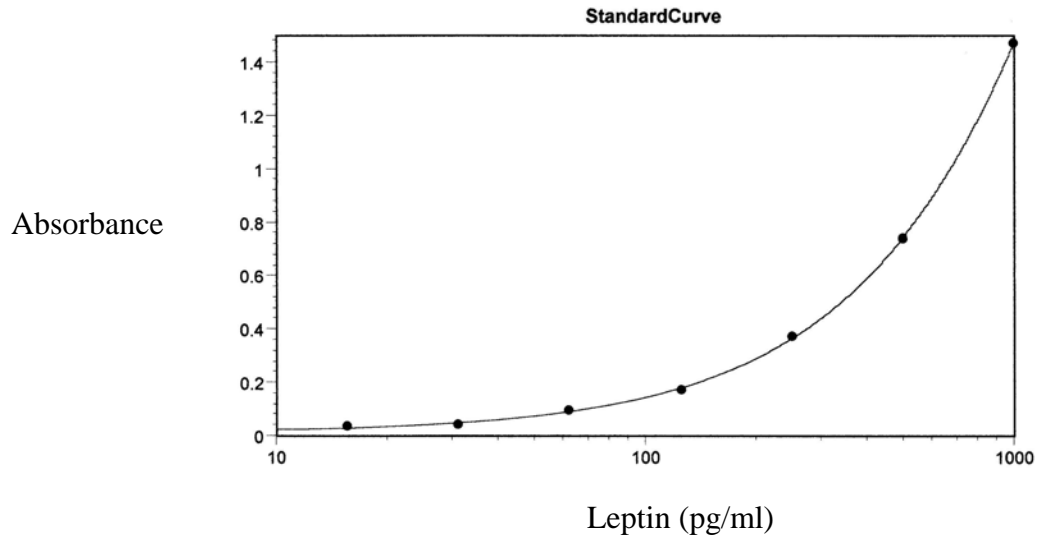


Human Leptin EIA Analysis



Plasma Required: 10-20 μ l
Range of Values Observed: 0.8 - 42.7 ng/ml

Human Leptin is measured utilizing a human Leptin Immunoassay kit purchased from R & D Systems, Minneapolis, MN (Catalogue #DLP00). The assay employs a quantitative sandwich enzyme immunoassay technique utilizing a monoclonal antibody specific for leptin precoated into wells of a 96 well microplate. Standards and samples are added to wells resulting in the binding of leptin to the immobilized antibody. After washing to remove any unbound substances. A mouse monoclonal antibody against leptin which is conjugated to horseradish peroxidase is added. After removal of unbound conjugate a substrate solution is added to each well and color allowed to develop proportionate to the amount of leptin present. The color development is stopped with the addition of 2 N H_2SO_4 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 and adjusted for any dilution of plasma.