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Method:	Radioimmunoassay (RIA)
Kit Manufacturer:	Millipore, St. Charles, MO
Description:	The Millipore Sensitive Human Leptin assay utilizes 125I-labeled Human Leptin and a Sensitive Human Leptin antiserum to determine the level of Leptin in serum, plasma or tissue culture media by the double antibody/PEG technique. In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A calibration or standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

Collection and Performance Characteristics

Tube type:	Preferred: SST Alternate: EDTA
Minimum Volume:	0.6 mL
Lowest Reportable Value:	0.78 ng/mL
Dynamic range	0.78-100 ng/mL
Precision	Intra assay variation 5.2-7.5% Inter assay variation 3.2-8.9%
Reference Range:	Leptin levels are directly correlated with degree of adiposity. Mean Leptin Values (BMI, body mass index ranges 18-25): Lean Males: 2.3 – 5.3 ng/mL Lean Females: 3.7 – 11.1 ng/mL Levels rise approximately 2.5 times faster in women per unit BMI as compared to men.