

Technical Bulletin

Changes in format for Leptin (rat) EIA Kit
Catalog Number: 900-015 / 900-015A

Effective Date: July, 2008

Please note that as of the effective date above the antibodies, standard, and format of the Leptin (rat) EIA Kit (Cat. No. 900-015) will be changed and the product given a new catalog number (Cat. No. 900-015A) and lower price. The new format requires less plate washing and offers more convenient incubation at room temperature. In addition, standards have been reformulated; we recommend that any internal controls be reevaluated. Extensive validation studies have been completed, which are summarized below.

Assay Procedure Comparison

Old Format (900-015) rat Leptin EIA Kit	New Format (900-015A) rat Leptin EIA Kit
Add 100 μ L standard & sample to the wells	Add 100 μ L standard & sample to the wells
Incubate 1 hour at 37°C	Incubate for 1 hour at RT, shaking
Wash 7 times	Wash 3 times
Add 100 μ L of antibody to the wells	Add 100 μ L of antibody to the wells
Incubate for 30 minutes at 37°C	Incubate for 1 hour at RT, shaking
Wash 9 times	Wash 3 times
Add 100 μ L of substrate to the wells	Add 100 μ L of conjugate to the wells
Incubate for 30 minutes at RT, in the dark	Incubate for 30 minutes at RT
Add 100 μ L stop solution	Wash 3 times
Read plate at 450 nm	Add 100 μ L of substrate to the wells
	Incubate for 30 minutes at RT
	Add 100 μ L stop solution
	Read plate at 450 nm

Assay Specification Comparison

Parameter	Old Format (900-015)	New Format (900-015A)
Standard Range	56 - 3,600 pg/mL	100 - 6,400 pg/mL
Sensitivity	46.7 pg/mL	67.2 pg/mL
Time to Answer	2 hours	3 hours
Incubation Conditions	37°C and RT	RT, shaking
Standard	Lyophilized	Lyophilized
Storage	4°C	4°C
Capture Antibody	Polyclonal	Polyclonal
Detection Antibody	Polyclonal	Polyclonal
Sample Type & Dilution	Culture supernates (\geq 1:2), serum (\geq 1:8), and EDTA plasma (\geq 1:8)	Culture supernates (none or 1:2), serum (1:4), heparin plasma (1:4), and EDTA plasma (1:4)
Cross Reactivity	rat Leptin 100%, mouse Leptin 17.9%, human Leptin 0.2%	rat Leptin 100%, mouse Leptin 318%, human Leptin 0.5%

Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity is observed. Non-conditioned Dulbecco's Modified Eagle's Medium (DMEM) with or without 10% fetal bovine serum (FBS) was spiked with recombinant leptin and diluted in the assay buffer. The assay buffer was spiked to the same concentration and used as a control to determine linearity of the culture medium. Pools of natural rat serum, heparin plasma, and Na EDTA plasma were also diluted in the assay buffer to produce values within the dynamic range of the assay.

Average % of Expected					
Dilution	DMEM	DMEM + 10% FBS	Serum	Heparin Plasma	EDTA Plasma
Neat	23	85	73	52	57
1:2	103	106	84	81	72
1:4	104	99	94	96	86
1:8	---	---	97	104	98
1:16	---	---	98	110	101

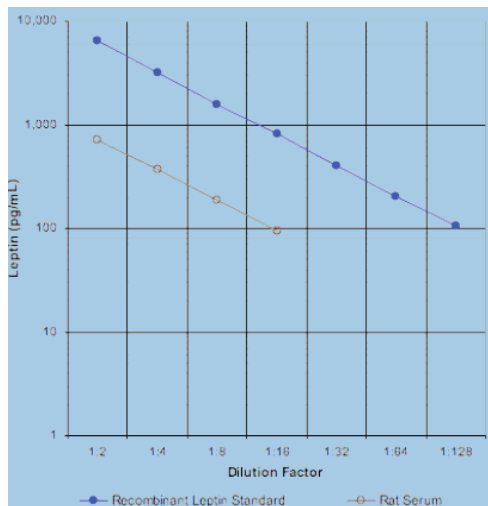
Comparison of Formats

Natural samples were quantified in both assay formats. The results, shown below, indicate that a factor should be applied to samples run in the revised kit (i.e. Old Format results multiplied by the Mean Factor for your sample type = New Format quantitation).

Benchmark of New Format vs. Old Format Rat Leptin EIA Kits			
Sample Type (# unique samples)	Mean New Format pg/mL (range)	Mean Old Format pg/mL (range)	Mean Factor New/Old (range)
Serum (n=6)	1824 (1228-2316)	2715 (2090-3662)	0.69 (0.48-0.90)
Na EDTA Plasma (n=6)	2558 (2048-2886)	9033 (7524-10697)	0.29 (0.24-0.33)
Na Heparin Plasma (n=5)	2205 (1588-2634)	7255 (5968-8215)	0.3 (0.27-0.32)
Recombinant Rat Leptin (n=2)	n/a	n/a	0.33 (0.32-0.33)

Parallelism

A parallelism experiment was carried out to determine if the recombinant leptin standard accurately determines leptin concentrations in biological matrices. To assess parallelism, values for rat serum were obtained from a standard curve using four parameter logistic curve fitting. The observed concentration was plotted against the dilution factor. Parallelism of the curves demonstrates that the antibody binding characteristics are similar enough to allow the accurate determination of analyte levels in diluted samples.



Please review the revised package insert carefully before use. We appreciate your continued business and welcome your comments or questions. technical@assaydesigns.com