DBC
Diagnostics Biochem Canada

Leptin ELISA

REF CAN-L-4260

ASSAY PROCEDURE



Bring kit components to room temperature. Prepare working solutions.


Pipette 20 µL of each calibrator, control and specimen sample.


Pipette 80 µL of the monoclonal anti-leptin-biotin conjugate into each well.


Incubate on a plate shaker for 1 hour at room temperature.


Wash 3 times.


Pipette 100 µL of streptavidin-HRP conjugate into each well.


Incubate on a plate shaker for 30 minutes at room temperature.



Wash 3 times. Pipette 100 µL of TMB substrate.


Incubate on a plate shaker for 10–15 minutes at room temperature.


Pipette 50 µL of stopping solution.


Read the plate on a microplate reader at 450 nm.

Human Leptin is a 16 kDa, 146 amino acid residue, non-glycosylated polypeptide. Leptin is encoded by the OB gene. Its major source is the adipose tissue, and its circulating concentrations indirectly reflect body fat stores.

Plasma or serum concentrations of leptin are increased in obese humans and strongly correlate with the degree of adiposity as expressed by percentage of body fat or body mass index.

The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure.

Many areas of leptin physiology remain to be investigated. The roles of leptin in metabolism, insulin sensitivity, as a potential therapeutic modality for weight loss as well as involvement in endocrine function are active areas of research. While the future for leptin as a therapeutic agent is not clear, its involvement in many areas of physiology undoubtedly makes this a new hormone which requires extensive study in the future to understand its physiology.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) for Leptin is 0.50 ng/mL, as determined by use of a NCCLS protcol and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 82 blank determinations; LoB=0.42 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following substances were tested at 1000 ng/mL and exhibited no cross-reactivity: Mouse Leptin, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF.

INTRA-ASSAY PRECISION

Four serum samples were assayed twenty times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.45	0.09	3.7
2	7.94	0.34	4.3
3	11.67	0.64	5.5
4	27.51	1.37	5.0

INTER-ASSAY PRECISION

Four samples were assayed ten times over a period of ten days. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	2.71	0.16	5.9
2	8.24	0.48	5.8
3	12.01	0.82	6.8
4	24.98	1.45	5.8

COMPARATIVE STUDIES

The DBC Leptin ELISA (DBC) was compared against a leading competitor's Leptin EIA kit (Kit X).

Thirty-eight serum samples ranging from 1.05–75.62 ng/mL were assayed with both kits, yielding the following results: Regression:
Kit X = 0.9644 (DBC) + 1.5489
r = 0.98
Kit X Mean: 21.13
DBC Mean: 20.30

RECOVERY

Spiked samples were prepared by adding defined amounts of leptin to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	3.89	-	-
+ 3.06	6.28	6.95	90.4
+ 8.06	10.98	11.95	91.9
+ 23.06	25.43	26.95	94.4
2 Unspiked	7.89	-	-
+ 1.06	8.82	8.95	98.5
+ 6.06	15.03	13.95	107.7
+ 21.06	30.32	28.95	104.7
3 Unspiked	11.61	-	-
+ 4.2	15.71	15.81	99.4
+ 12.8	25.42	24.41	104.1
+ 29.46	41.18	41.07	100.3

LINEARITY

Three patient serum samples were serially diluted with leptin assay buffer. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	3.03	-	-
1:2	1.42	1.52	93.4
1:4	0.71	0.76	93.4
1:8	0.35	0.38	92.1
2	11.27	-	-
1:2	5.93	5.64	105.1
1:4	3.05	2.82	108.2
1:8	1.35	1.41	95.7
3	27.91	-	-
1:2	14.91	13.96	106.8
1:4	6.74	6.98	96.6
1:8	3.29	3.49	94.3

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (ng/mL)	Range (ng/mL)
Lean Women	7.4	3.7–11.1
Lean Men	3.8	2.0–5.6

Leptin values are approximately 2.5 times higher in women than men per unit BMI.

Ordering Information: REF CAN-L-4260

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