

PRODUCT INFORMATION & MANUAL

Human Leptin Instant ELISA

BMS2039INST

Enzyme-linked immunosorbent assay for
quantitative detection of human Leptin.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests



*Human Leptin
Instant ELISA*

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1 Intended Use

The human Leptin Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human Leptin. **The human Leptin Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures**

2 Summary

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including the regulation (decrease) of appetite and (increase) of metabolism. The Ob(Lep) gene (Ob for obese and Lep for leptin) is located on chromosome 7 in humans. Leptin binds to the Ventral Medial nucleus of the hypothalamus, known as the satiety center. Binding of leptin to this nucleus signals to the brain that the body has had enough to eat. Thus, circulating leptin levels give the brain a reading of energy storage for the purposes of regulating appetite and metabolism. Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and by increasing the activity of neurons expressing α -melanocyte-stimulating hormone (α -MSH). The NPY neurons are a key element in the regulation of appetite; small doses of NPY injected into the brains of experimental animals stimulates feeding, while selective destruction of the NPY neurons in mice causes them to become anorexic. Conversely, α -MSH is an important mediator of satiety, and differences in the gene for the receptor at which α -MSH acts in the brain are linked to obesity in humans.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

An anti-human Leptin coating antibody is adsorbed onto microwells. Human Leptin present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human Leptin antibody binds to human Leptin captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human Leptin.

Following incubation unbound biotin conjugated antihuman Leptin and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of soluble human Leptin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human Leptin standard dilutions and human Leptin sample concentration determined.

Figure 1

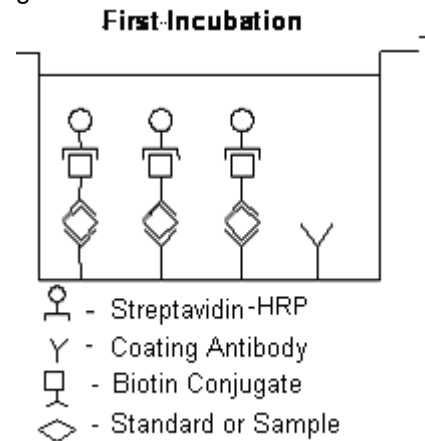


Figure 2

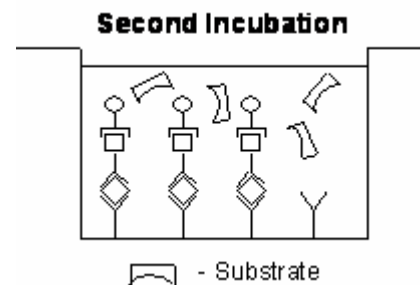
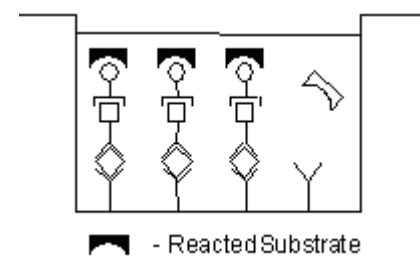


Figure 3



4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human Leptin, **Biotin-Conjugate** (anti-human Leptin monoclonal antibody), **Streptavidin-HRP** and Assay Buffer, lyophilized
- 2 aluminium pouches with a human Leptin **Standard curve** (coloured)
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (5 ml) **Assay Buffer Concentrate 20x** (Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Serum and plasma (EDTA, citrate, heparinized) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human Leptin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 μ l and 500 μ l) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

Buffer concentrates should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in **buffer concentrates**, warm them gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

9.2 Assay Buffer

Pour the entire contents (5ml) of the **Assay Buffer Concentrate** into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
 - **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
 - **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
 - **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
 - **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
 - **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**
- a. Prepare your samples before starting with the test procedure. Dilute serum or plasma samples 1:25 with **Assay Buffer** according to the following dilution scheme:
10 µl sample + 240 µl Assay Buffer
 - b. Determine the number of Microwell Strips required to test the desired number of samples plus Microwell Strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra Microwell Strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place Microwell Strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
 - c. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).
 - d. Add 100 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (4000 pg/ml)	Standard 1 (4000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2000 pg/ml)	Standard 2 (2000 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1000 pg/ml)	Standard 3 (1000 pg/ml)	Sample 3	Sample 3
D	Standard 4 (500 pg/ml)	Standard 4 (500 pg/ml)	Sample 4	Sample 4
E	Standard 5 (250 pg/ml)	Standard 5 (250 pg/ml)	Sample 5	Sample 5
F	Standard 6 (125 pg/ml)	Standard 6 (125 pg/ml)	Sample 6	Sample 6
G	Standard 7 (63 pg/ml)	Standard 7 (63 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- e. Add 50 µl of each 1:25 prediluted **sample**, in duplicate, to the **designated wells** and mix the contents.
- f. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker at 400 rpm.
- g. Remove adhesive film and empty wells. **Wash** the microwell strips 6 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

- h. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- i. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

- j. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

- k. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human Leptin standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human Leptin concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human Leptin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human Leptin concentration.
- ***Samples have been diluted 1:50, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 50).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human Leptin levels. Such samples require further external predilution according to expected human Leptin values with Assay Buffer (1x) in order to precisely quantitate the actual human Leptin level.**
- It is suggested that each testing facility establishes a control sample of known human Leptin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 μl to the final volume per well. These 100 μl are composed of 50 μl of Sample Diluent plus 50 μl of the 1:25 prediluted sample. This is a 1:50 dilution.

The remaining 50 μl to give 150 μl are due to the addition of 50 μl conjugate to all wells.

50 μl Sample Diluent and 50 μl conjugate results in 100 μl reconstitution volume, addition of 50 μl 1:25 prediluted sample (50 μl + 50 μl 1:25 prediluted sample = 1:50 dilution)

Figure 4

Representative standard curve for human Leptin Instant ELISA. Human Leptin was diluted in serial 2-fold steps in Assay Buffer. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

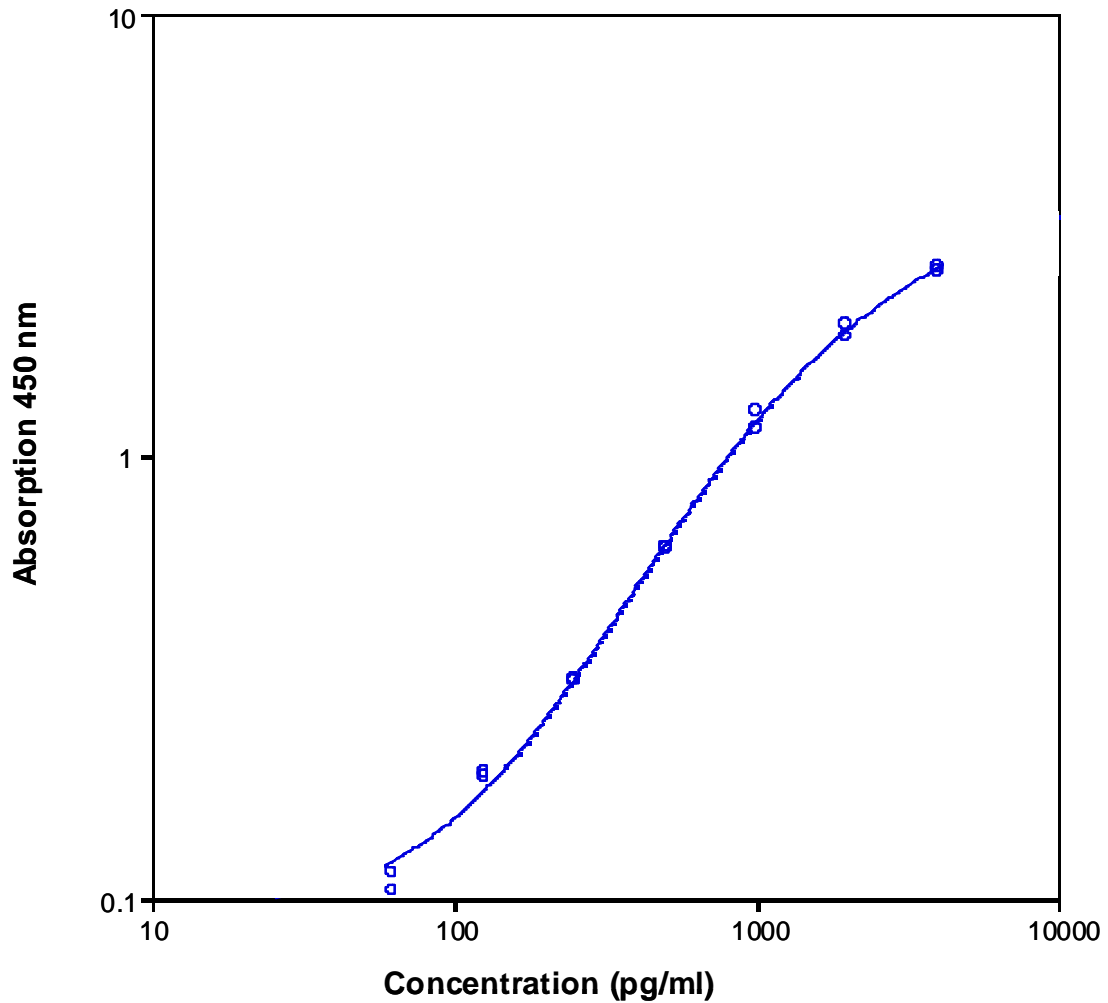


Table 2

Typical data using the human Leptin INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human Leptin Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	4000	2.683 2.622	2.652	1.6
2	2000	1.853 1.968	1.911	4.3
3	1000	1.150 1.257	1.203	6.3
4	500	0.614 0.621	0.618	0.8
5	250	0.310 0.315	0.313	1.1
6	125	0.193 0.189	0.191	1.6
7	63	0.115 0.104	0.109	7.4
Blank	0	0.044 0.050	0.047	9.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human Leptin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 20 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different natural levels of human Leptin. 2 standard curves were run on each plate. Data below show the mean human Leptin concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 5.7%.

Table 3

The mean human Leptin concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human Leptin Concentration (pg/ml)	Coefficient of Variation (%)
1	1	59210	1.9
	2	57051	2.9
	3	57233	1.9
2	1	89608	3.5
	2	88294	2.0
	3	85028	2.1
3	1	23739	11.7
	2	28364	3.5
	3	29049	2.2
4	1	8839	13.6
	2	10774	7.8
	3	10661	7.1
5	1	20568	12.2
	2	23033	4.6
	3	19829	5.3
6	1	8989	10.9
	2	7930	4.9
	3	9120	5.0

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human Leptin. 2 standard curves were run on each plate. Data below show the mean human Leptin concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.9%.

Table 4

The mean human Leptin concentration and the coefficient of variation of each sample

Sample	Mean human Leptin Concentration (pg/ml)	Coefficient of Variation (%)
1	57832	2.1
2	87643	2.7
3	27051	10.7
4	10091	10.8
5	21143	7.9
6	8680	7.5

13.3 Spike Recovery

The spike recovery was evaluated by spiking 2 levels of human Leptin into samples. Recoveries were determined in 4 independent experiments with 6 replicates each.

The amount of endogenous human Leptin in unspiked samples was subtracted from the spike values.

Recovery ranged from 42% to 129% with an overall mean recovery of 86% (see Table 5).

Table 5

Sample matrix*	Spike high (%)	Spike medium (%)
Serum	84	68
Plasma (EDTA)	103	87
Plasma (citrate)	86	105
Plasma (heparin)	81	70

*Due to high endogen Leptin levels data for low spikes are not indicated.

13.4 Dilution Parallelism

Serum and plasma samples (4 samples of each sample matrix) with different levels of human Leptin were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 87% and 92% with an overall recovery of 90% (see Table 6).

Table 6

Sample Matrix	Recovery of Exp. Val. (%)
Serum	92
Plasma (EDTA)	87
Plasma (citrate)	97
Plasma (heparin)	91

Table 7 shows detailed recovery data of 4 serum samples.

Table 7

Sample	Dilution	Mean human Leptin Concentration (pg/ml)		% Recovery of Exp. Val.
		Expected Value	Observed Value	
1	1		54504	
	2	27252	25152	92.3
	4	13626	11482	84.3
	8	6813	5449	80.0
2	1		85031	
	2	42516	40833	96.0
	4	21258	19986	94.0
	8	10629	9520	89.6
3	1		28093	
	2	14046	12624	89.9
	4	7023	5626	80.1
	8	3512	2812	80.1
4	1		25307	
	2	12654	11740	92.8
	4	6327	5314	84.0
	8	3164	2857	90.3

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human Leptin levels determined. There was no significant loss of human Leptin immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human Leptin level determined after 24 h. There was no significant loss of human Leptin immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human Leptin. The interference of circulating factors of the immune syteme was evaluated by spiking these proteins at physiologically relevant concentrations into positive serum. There was no crossreactivity detected.

13.7 Expected Values

A panel of 40 sera samples from randomly selected apparently healthy donors (males and females) was tested for human Leptin.

The detected human Leptin levels ranged between 797 and 508072 pg/ml with a mean level of 12991 pg/ml. The levels measured may vary with the sample collection used.

For detected human Leptin levels see Table 8.

Table 8

Sample Matrix	Number of Samples > S7*	Detectable Samples			
		Minimum (pg/ml)	Maximum (pg/ml)	Mean (pg/ml)	Standard Deviation (pg/ml)
Serum	22	1058	45881	9442	10338
Plasma (EDTA)	18	894	50376	14967	15423
Plasma (Citrate)	29	797	38246	9740	10344
Plasma (Heparin)	26	890	58072	17813	15863

*S7 = lowest standard point

14 Ordering Information

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15 Reagent Preparation Summary

15.1 Wash Buffer

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

15.2 Assay Buffer

Add **Assay Buffer Concentrate** 20 x (5 ml) to 95 ml distilled water

16 Test Protocol Summary

- Predilute sample 1:25 with Assay Buffer
- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 μ l distilled water to sample wells.
- Add 50 μ l 1:25 prediluted sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) if available on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 6 times with 400 μ l Wash Buffer.
- Add 100 μ l of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 μ l Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:50, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 50)