



HUMAN LEPTIN ENZYME IMMUNOASSAY KIT

catalogue # A05174

96 wells

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*For research laboratory use only.
Not for diagnostic use.*



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HUMAN LEPTIN EIA KIT

96 wells
Storage: 2-8 °C
Expiry date: stated on the package

This kit contains:

- ☞ A covered 96 well plate, precoated with a polyclonal anti-Leptin antibody, ready to use
- ☞ One vial of anti-Leptin tracer, ready to use
- ☞ Six vials of human Leptin standards (1, 2, 5, 10, 20, 50 ng/mL), ready to use
- ☞ Two vials of Quality Controls: Low and High, lyophilised
- ☞ One vial of Substrate (TMB) solution, ready to use
- ☞ One vial of Stop solution (0.2 M H₂SO₄), ready to use
- ☞ One vial of EIA buffer, ready to use.
- ☞ One vial of concentrated Wash buffer (10x), liquid
- ☞ One instruction booklet
- ☞ One template sheet
- ☞ One well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample or reagent and dispense it back into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.

Not for diagnostic use.

Do not pipet liquids by mouth.

Do not use kit components beyond the expiration date.

Do not mix different lot numbers

Do not eat, drink or smoke in area in which kit reagents are handled.

Avoid splashing.

This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. Wear gloves and laboratory coats are recommended when handling immunodiagnostic materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

PRINCIPLE OF THE ASSAY

Leptin, the product of the ob (obese) gene, is produced mainly in the adipose tissue, and is considered to play an important role in appetite control, fat metabolism and body weight regulation. The primary effect of leptin appears to be mediated by leptin receptors expressed mainly in the hypothalamus. In humans, leptin levels correlate with body mass index (BMI) and percentage body fat, and are elevated even in obese individuals. Leptin has a dual action; it decreases the appetite and increases energy consumption. Leptin is secreted in circadian fashion with nocturnal rise in both lean and obese patients.

Mutations of the ob gene resulting in leptin deficiency are the cause of obesity in the ob/ob mice suggesting that endogenous leptin can normalize their body weight. In contrast, human obese subjects may have high level of leptin, indicating a mechanism of leptin resistance.

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a polyclonal antibody specific of human leptin. This antibody will bind any Leptin introduced in the wells (sample or standard).

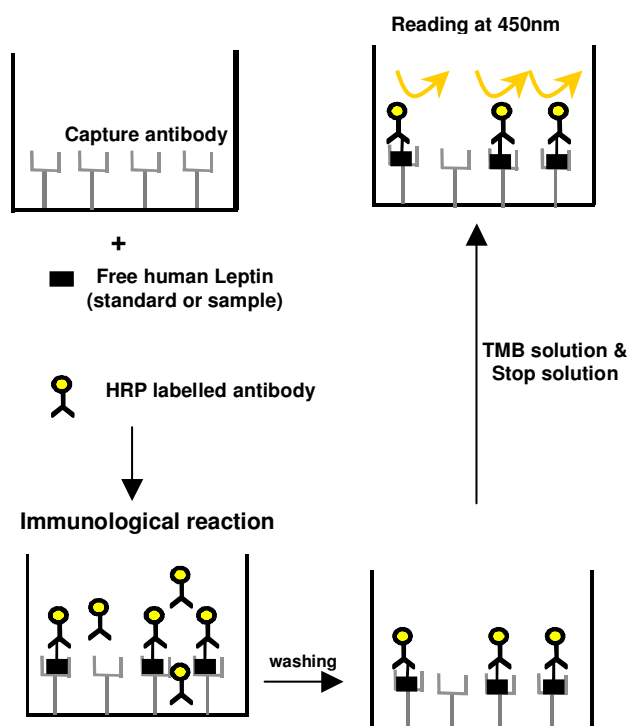
An horseradish peroxidase (HRP) conjugated polyclonal antibody which binds selectively to different epitopes on the leptin molecule, is also added to the wells.

This allows the two antibodies to form a sandwich by binding on different parts of the human leptin molecule.

The sandwich is immobilised on the plate so the excess reagents may be washed away. The concentration of the human leptin is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulfuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the human leptin present in the well during the immunological incubation.

The principle of the assay is summarised below:



MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR THE ASSAY

- ☞ Precision micropipettes (50 to 200 μ L)
- ☞ Spectrophotometer plate reader (450 nm \pm 10 nm filter)
- ☞ Microplate washer (or washbottles)
- ☞ Microplate shaker
- ☞ Multichannel pipette 100 μ L and disposable tips
- ☞ Distilled or deionised water
- ☞ Polypropylene tubes



SAMPLE PREPARATION

This assay may be used to measure human leptin in human samples such as serum, plasma, and culture supernatant.

GENERAL PRECAUTIONS

- ☞ All samples must be free of organic solvents prior to assay.
- ☞ Samples should be assayed immediately after collection or should be stored frozen.

SAMPLE PREPARATION

- ☞ No prior extraction procedure is necessary.
- ☞ To measure human Leptin, dilute samples 1/3 in EIA buffer (i.e. 100 μ L sample + 200 μ L EIA buffer).

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Quality Controls and the Wash buffer.

- ☞ Human Leptin standard
 - Dilute standards 1/3 in EIA buffer prior to use (i.e. 100 μ L standard + 200 μ L EIA buffer).
 - Do not store the diluted standards.
- ☞ Quality Controls
 - Reconstitute each vial of Quality Control with 350 μ L of distilled or deionised water at least 30 minutes prior the assay.
 - Dilute Quality Controls 1/3 in EIA buffer (i.e. 100 μ L QC + 200 μ L EIA buffer).
 - Do not store diluted Quality Controls.
- ☞ Wash buffer
 - Dilute 100 mL of concentrated Wash buffer to 1000 mL with distilled or deionised water.
- ☞ Hydrogen peroxide/TMB solution
 - Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on the following page. The contents of each well may be recorded on the sheet provided with the kit.

PIPETTING THE REAGENTS

All samples and reagents must reach room temperature prior to performing the assay. Use different tips to pipet the buffer, standard, sample, tracer, antiserum and other reagents.

12	*	*	*	*	*	*	*	*
11	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*
9	*	*	*	*	*	*	*	*
8	*	*	*	*	*	*	*	*
7	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	*
5	*	*	*	*	*	*	*	*
4	*	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*
2	S4	S4	S5	S5	S6	S6	*	*
1	B	B	S1	S1	S2	S2	S3	S3
	A	B	C	D	E	F	G	H

B: Blank

S1-S6: Standards 1-6

*: Samples or Quality Controls.

- ↪ Human Leptin standard:
Dispense 100 µL of the six standards (S1 to S6), in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.
- ↪ Quality Control and samples:
Dispense in duplicate 100 µL of diluted Quality Control and samples to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.
- ↪ EIA buffer:
Dispense in duplicate 100 µL to the blank (B) wells.

INCUBATING THE PLATE

- ↪ Cover the plate with adhesive film and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- ↪ Rinse each well 3 times with the Wash buffer (350 µL/ well). Slightly shake the plate for 5 minutes (with orbital shaker).
- ↪ Anti-Leptin tracer:
Dispense 100 µL to each well.
- ↪ Cover the plate with adhesive film and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- ↪ Rinse each well 3 times with the Wash buffer (350 µL/ well). Slightly shake the plate for 5 minutes (with orbital shaker).

DEVELOPING AND READING THE PLATE

- ↪ Dispense 100 µL of Substrate solution to the 96 wells. Incubate the plate in the dark during 10 to 15 minutes at room temperature. Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil
- ↪ Stop the colour development by adding 100 µL of Stop solution.
- ↪ Read the absorbance at 450 nm within 5 minutes following stop solution addition.

*Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Leptin concentration of off-scale samples. . **The readings at 405 nm should not replace the on-scale readings at 450 nm***

Enzyme Immunoassay Protocol (Volumes are in μL)				
	Blank	Standard	Sample	
EIA Buffer	100	-	-	
Standard	-	100	-	
Sample	-	-	100	
Incubate the plate at room temperature for 1 hour				
Wash the plate 3 times				
Tracer	-	100	-	100
Incubate the plate at room temperature for 1 hour				
Wash the plate 3 times				
TMB solution	100	100	-	100
Incubate the plate in the dark at room temperature during 10-15 minutes				
Stop solution	100	100	-	100
Read the plate at 450 nm				

DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- ↳ Using a semi-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.
- ↳ To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- ↳ Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

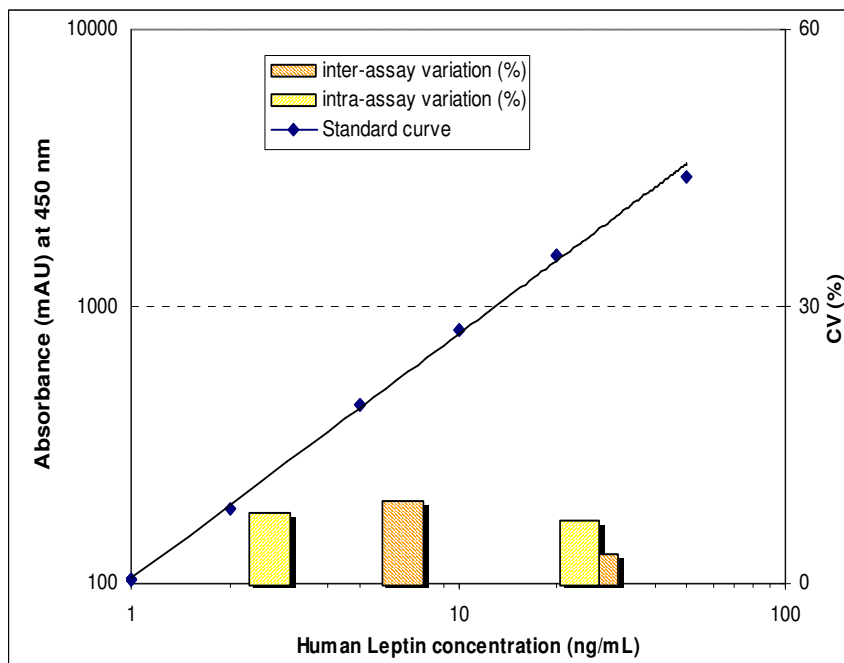
The following data are for demonstration purposes only. Your data may be different but still correct. These data were obtained using all reagents supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human Leptin	mAU
Standard 50 ng/mL	2 921
Standard 20 ng/mL	1 520
Standard 10 ng/mL	825
Standard 5 ng/mL	442
Standard 2 ng/mL	186
Standard 1 ng/mL	103
Blank	20
QC High	1 578
QC Low	459

ACCEPTABLE RANGE

QC samples: see label on the vials.

HUMAN LEPTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of human Leptin has been validated for its use in human serum, human plasma and culture supernatant.

☞ Cross-reactivity:

Mouse Leptin	<0.1%
Rat Leptin	<0.1%
Bovine Leptin	<0.1%
Rabbit Leptin	<0.1%
Horse Leptin	<0.1%
Goat Leptin	<0.1%
Sheep Leptin	<0.1%
Pig Leptin	<0.1%

☞ Sensitivity:

The limit of detection is 0.5 ng/mL (defined as such a concentration of human Leptin giving absorbance lower than mean absorbance of blank minus three standard deviations of the absorbance of blank: $A_{\text{blank}} - 3 \cdot SD_{\text{blank}}$).

The EIA Buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞ Precision:

• Intra-assay variation (n=8)

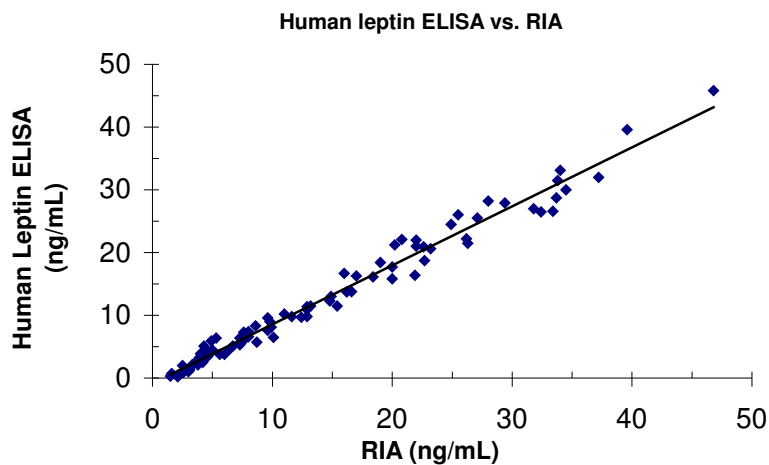
Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	3.54	0.27	7.5
2	13.63	0.41	3.0
3	25.44	1.38	5.4
4	25.58	1.68	6.7

• Inter-assay variation (n=6)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	5.41	0.50	9.2
2	8.65	0.68	7.8
3	13.9	0.95	6.8
4	25.12	0.81	3.2

☞ Method comparison:

The SPI-BIO's Human Leptin EIA was compared to other commercial immunoassays, measuring 77 or 68 serum samples, in radioimmunoassay (RIA).



☞ Recovery test:

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	7.89	-	-
	10.14	11.69	86.7
	15.12	16.47	91.8
	22.17	24.14	91.8
2	12.95	-	-
	18.26	16.75	109.0
	19.55	21.53	90.8
	27.20	29.20	93.2
3	8.09	-	-
	10.92	12.53	87.2
	14.31	16.43	87.1
	20.37	23.03	88.4
2	13.84	-	-
	13.68	18.28	89.6
	18.08	22.18	81.5
	26.71	28.78	92.8

☞ Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	13.27	-	-
	1/2	7.62	6.64	114.8
	1/4	3.76	3.32	113.3
	1/8	1.58	1.66	96.3
2	-	15.49	-	-
	1/2	8.39	7.75	108.3
	1/4	3.93	3.87	101.5
	1/8	2.31	1.94	119.3
3	-	15.23	-	-
	1/2	7.78	7.62	102.2
	1/4	3.68	3.81	96.7
	1/8	1.84	1.90	96.7
4	-	27.76	-	-
	1/2	13.69	13.88	98.6
	1/4	6.87	6.94	99.0
	1/8	4.10	3.47	118.2



ASSAY TROUBLE SHOOTING

- ☞ Absorbance values too low:
 - One reagent has not been dispensed
 - Incorrect preparation or reagent storage
 - Assay performed before reagents reach room temperature
- ☞ High signal and background in all wells:
 - Inefficient washing
 - Overdeveloping; incubation time should be reduced before adding Stop Solution
- ☞ High dispersion of duplicates:
 - Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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