LEPTIN
(human)

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LEPTIN (human)
Enzyme Immunoassay kit
#A05174.96 wells

For research laboratory use only
Not for human diagnostic use

Fabriqué en France
Made in France

#A11174
Version: 0120

Table of contents

96 wells

Storage: +4°C

Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
Leptin (human) precoated microtiter Plate	A08174	1	Ready to use
Leptin (human) tracer	A40174	13mL	Liquid
Leptin (human) Standard	A06174	2 vials	Lyophilized
Leptin (human) Quality Control HIGH	A10174_H	2 vials	Lyophilized
Leptin (human) Quality Control LOW	A10174_L	2 vials	Lyophilized
ELISA Buffer	A07010	13mL	Liquid
Concentrated Wash Buffer (10x)	A17012	100mL	Liquid
Substrate Solution (TMB)	A09010	13mL	Liquid
Stop Solution	A22000	13mL	Liquid
Technical Booklet	A11174	1	-
Well cover Sheet	-	1	-

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 39 samples in duplicate.

Precaution for use

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

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area in which kit reagents are handled
- Avoid splashing

HRP Stop Solution and HRP Substrate Solution are harmful solutions. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

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.

This kit contains components of animal origin. These materials should be handled as potentially infectious

Wear gloves and laboratory coats are recommended when handling immunodiagnostic materials and samples of human origin.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

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 endogeneous leptin can normalize their body weight. In contrast, human obese subjects may have high level of leptin, indicating a mechanism of leptin resistance.

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to Leptin (human).

Leptin (human) introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a second polyclonal antibody tagged with HRP also specific for Leptin (human).

The two antibodies then form a sandwich by binding on different parts of the Leptin molecule.

The sandwich is immobilised on the plate so reagents in excess may be washed away.

The concentration of Leptin (human) is determined by measuring the enzymatic activity of immobilized tracer using TMB. The tracer acts on TMB to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry at 450 nm, is proportional to the amount of Leptin (human) present in the well during the immunological incubation.

Assay characteristics

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

- ***Limit of detection (LOD):*** is 0.2 ng/mL (defined as such a concentration of human Leptin giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3*SD_{blank}).
  The ELISA Buffer was pipetted into blank wells.

- ***Cross-reactivity***

Molecule/Species	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%
Cat Leptin	<0.1%
Dog Leptin	<0.1%

Hamster Leptin	<0.1%
Monkey Leptin	<0.1%

> *Precision*

- Intra-assay (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	15.01	0.06	4.2
2	3.56	0.02	7.6

- Inter-assay (Run to Run; n=6)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	15.39	1.04	6.7
2	29.34	1.28	4.4

> *Recovery test (serum samples)*

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	4.22	-	-
	7.52	8.40	89.5
	11.85	13.37	88.6
	17.41	17.76	98.0
2	14.09	-	-
	17.78	18.27	97.3
	19.92	23.24	85.7
	25.91	27.63	93.8

> *Dilution test (serum samples)*

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	20.99	-	-
	1:2	10.34	10.49	98.5
	1:4	5.32	5.25	101.4
	1:8	2.45	2.62	93.4
2	-	29.94	-	-
	1:2	15.72	14.97	105.0
	1:4	7.80	7.49	104.2
	1:8	3.83	3.74	102.3

> *Serum / Plasma Samples*

Citrate, EDTA and heparin plasmas were compared to respective serum samples obtained from 9 individuals.

Sample	Mean (ng/mL)	Mean Plasma /Serum (%)	Coefficient of determination R^2
Serum	8.97	-	-
Citrate Plasma	7.22	80.6	0.97
EDTA Plasma	8.21	91.6	0.97
Heparin Plasma	9.19	102.7	0.96

Materials and equipment required

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

For the assay:

- Precision micropipettes (10 to 1000 µL)
- Spectrophotometer plate reader (450 nm filter) preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Microplate washer (or washbottles)
- Vortex mixer
- Orbital microplate shaker
- Multichannel pipette and disposable tips 100µL
- Deionized (distilled) water
- Polypropylene tubes
- Glassware (graduated cylinder and bottle) for ELISA buffer and Wash buffer

Sample preparation

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

It is the responsibility of the user to check the compatibility of the assay with the study matrix.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C or at -80°C prior the use with the assay. Avoid using hemolyzed or lipemic samples.
- No decline was observed in concentration of human leptin in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample preparation

No prior extraction procedure is necessary.

To measure human Leptin, dilute samples at 1:3 in ELISA Buffer (i.e. 100 µL sample + 200 µL ELISA Buffer), just prior the assay. Mix well, vortex is recommended.
Do not store diluted samples.

Reagent preparation

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 39 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of standard and one of each quality controls.

All reagents need to be brought to room temperature (around +20°C) prior to the assay.

Assay reagents are supplied ready to use, except the Standards, Quality Controls and the Wash buffer.
Stability after opening of Tracer, ELISA Buffer, substrate solution and stop solution: 3 months when stored at +4°C.

Leptin (human) Standard

Reconstitute the Leptin (human) Standard vial #A06174 with X µL of ELISA Buffer just prior the assay. The volume X is indicated on the vial of the standard. Let it dissolve at least 15 minutes with occasional gentle shaking. Do not make it foam.

The concentration of the leptin in the stock solution (S1) is 50 ng/mL.

Prepare five polypropylene tubes (for the 5 other standards) and add ELISA Buffer into each tube as indicated in the following table. Then prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of ELISA Buffer	Standard concentration
S1	-	X (see label)	50 ng/mL
S2	200 µL of S1	300 µL	20 ng/mL
S3	250 µL of S2	250 µL	10 ng/mL
S4	250 µL of S3	250 µL	5 ng/mL
S5	200 µL of S4	300 µL	2 ng/mL
S6	250 µL of S5	250 µL	1 ng/mL

Please note that the buffer volume X used to reconstitute S1 gives a standard that is diluted to 1:3. This is the ready to use concentration. Do not dilute it further.
Refer to data analysis chapter for more information.

Stability: Do not store the standard solutions S1 to S6.

▷ Quality Controls

Reconstitute each vial of Quality Control with 350 µL of distilled or deionised water at least 30 minutes prior the assay with occasional gentle shaking. Do not make them foam. Dilute reconstituted Quality Controls at 1:3 in ELISA Buffer (i.e. 100 µL QC + 200 µL ELISA Buffer).

Refer to the vial label for current QC concentration.

Do not store diluted Quality Controls.

▷ Wash Buffer

Dilute 100 mL of concentrated Wash buffer to a final volume of 1000 mL with distilled or deionised water.

Stability at +4°C: 1 month.

▷ Substrate solution (TMB)

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 following the instructions hereafter.

Plate preparation

Open the plate pouch and select the sufficient strips for your assay and place the unused strips back in the pouch.
Stability at +4°C: 3 months.

Plate set-up

A plate set-up is suggested hereafter. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

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

B : Blank
QC: Quality Controls
S1-S6 : Standards 1-6
* : Samples

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 and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

ELISA Buffer

Dispense 100 µL in blank (B) wells.

Leptin (human) Standard

Dispense 100 µL of each of the six standards (S6 to S1) in duplicate in appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

Leptin Quality Controls and Sample

Dispense in duplicate 100 µL of diluted Quality Controls and samples to appropriate wells. Highly concentrated samples may be diluted in ELISA Buffer.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with the Wash buffer (350 µL/ well). After last wash, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

> ***Leptin tracer***

Dispense 100 µL to each well.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with the Wash buffer (350 µL/ well). After last wash, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Developing and reading the plate

- Dispense 100 µL of Substrate solution in each well. Incubate the plate in the dark during 10 to 15 minutes at room temperature (10 minutes at 20°C or up to 20 minutes is temperature is lower than 20°C). 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. Preferably use a reference wavelength set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm.

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.***

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
	Blank	Standard	Sample or QC
ELISA Buffer	100	-	-
Standard	-	100	-
Sample or QC	-	-	100
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Tracer	100	100	100
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Substrate solution (TMB)	100	100	100
Incubate the plate in the dark during 10 to 15 minutes at room temperature			
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 wells from the absorbance readings of the rest of the plate. If not, do it at this step.

- Calculate the average absorbance for each standard, QC and sample.
- Using a semi-log scale, plot the absorbance on y axis versus the concentration on x axis for each standard. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample.

Please note that: Standards are diluted at 1:3 during reconstitution with the specified volume of ELISA Buffer. Samples and QCs are all diluted at 1:3 prior to analysis. Therefore, there is no need to take this dilution factor into account.

- Most plate readers are supplied with a curve-fitting software capable of graphing these data (logit/log or 4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Acceptable range

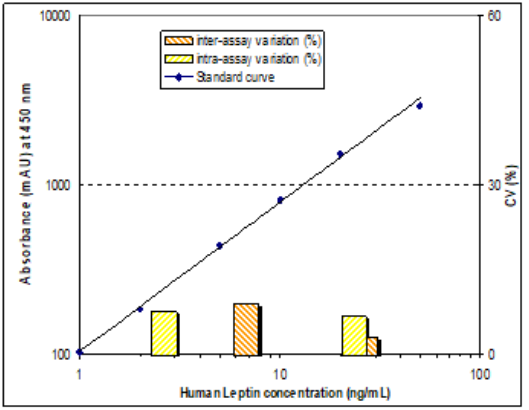
- QC ±25% of the expected concentration (see the label of the QC vial)

Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.
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.

Standard	Leptin (human) ng/mL	Absorbance A.U.
S1	50	2.921
S2	20	1.520
S3	10	0.825
S4	5	0.442
S5	2	0.186
S6	1	0.103
Blank		0.020
QC High		1.578
QC Low		0.459

Typical Leptin (human) standard curve



Troubleshooting

Absorbance values are too low:

- one reagent has not been dispensed,
- incorrect preparation,
- assay performed before reagents reached room temperature,
- reading time not long enough.

> ***High signal and background in all wells:***

- inefficient washing,
- overdeveloping (incubation time should be reduced),
- high ambient temperature.

> ***High dispersion of duplicates:***

- poor pipetting technique
- irregular plate washing.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent will be happy to assist you. Feel free to contact our Technical Support staff by phone +33 (0)139 306 036, fax +33 (0)139 306 299 or E-mail tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes ELISA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Technical Support.

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Haluzik M, Sindelka G, Widimsky J Jr, Prazny M, Zelinka T, Skrha J.: Serum Leptin levels in patients with primary hyperaldosteronism before and after treatment: relationships to insulin sensitivity. *J Hum Hypertens; 16(1):41-5 (2002)*

1 2 3 4 5 6 7 8 9 10 11 12
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With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control.
We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

Bertin Bioreagent has also a long expertise in developing customized solutions adapted to your need. Feel free to contact us for your special projects!

To offer a complete solution to researchers, Bertin Instruments offers a range of unique laboratory equipment from Air Sample collection, Sample Homogenisation and Digital Imaging.

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