# HUMAN LEPTIN

# ENZYME IMMUNOASSAY KIT

catalogue # A05174

96 wells

## TABLE OF CONTENTS



*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
- One vial of human Leptin standard (50 ng/mL), lyophilized
- Two vials of Quality Controls: Low and High, lyophilized
- One vial of Substrate (TMB) solution, ready to use
- One vial of Stop solution (0.2 M $H_2SO_4$), 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 endogeneous 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).

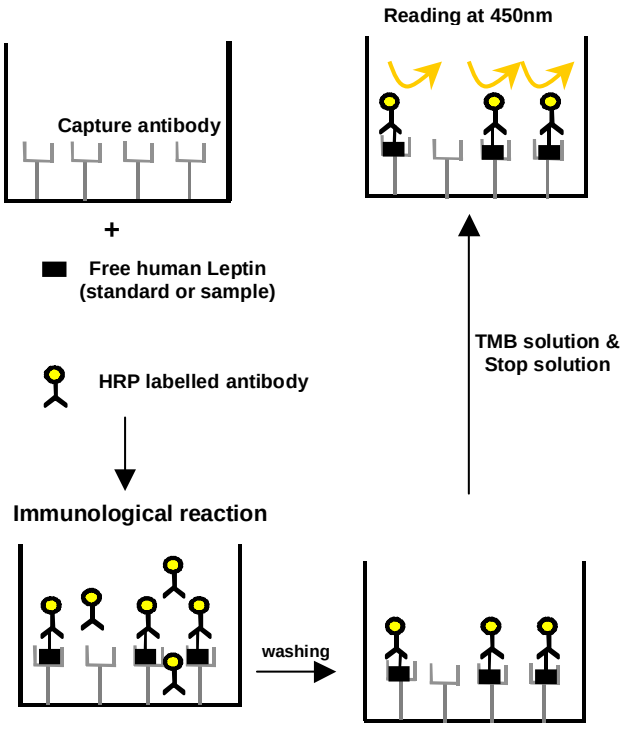
An horseradish peroxydase (HRP) conjugated polyclonal antibody which binds selectively to different epitopes on the leptin molecule, is also added to the wells.

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 sulphuric 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 µL)

☞Spectrophotometer plate reader (450 nm +/-10 nm filter)

☞Microplate washer (or washbottles)

☞Microplate shaker

☞Multichannel pipette 100 µL 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 µL sample + 200 µL EIA buffer). Mix well, vortex is recommended.

## REAGENT PREPARATION

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

☞Human Leptin standard

Reconstitute Leptin standard with X µL of EIA buffer just prior the assay. The volume X is indicated on the vial of the corresponding standard. Let it dissolve at least 15 minutes with gentle shaking.
The concentration of the leptin in the stock solution (S1) is 50 ng/mL.

Then prepare standards as follows:

| Volume of Standards | Added volume of EIA buffer | Concentration of reconstituted standards |
|---|---|---|
| / | X (see label) | S1 (50 ng/mL) |
| 200 µL of S1 | 300 µL | S2 (20 ng/mL) |
| 250 µL of S2 | 250 µL | S3 (10 ng/mL) |
| 250 µL of S3 | 250 µL | S4 (5 ng/mL) |
| 200 µL of S4 | 300 µL | S5 (2 ng/mL) |
| 250 µL of S5 | 250 µL | S6 (1 ng/mL) |

The reconstituted standard in stock solution has to be used immediately or to be stored frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the standard solutions S2 to S6.


The reconstitution volume dilutes standard 3x, the same as samples and Quality Controls. Ready to use, do not dilute them.

☞Quality Controls

Reconstitute each vial of Quality Control with 350 µL of distilled or deionised water at least 30 minutes prior the assay. Refer to the vial label for current QC concentration.
Dilute Quality Controls 1/3 in EIA Buffer (i.e. 100 µL QC + 200 µL 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.
Stable 1 month at +4°C

☞Hydrogen peroxide/TMB solution

Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

# ASSAY PROCEDURE

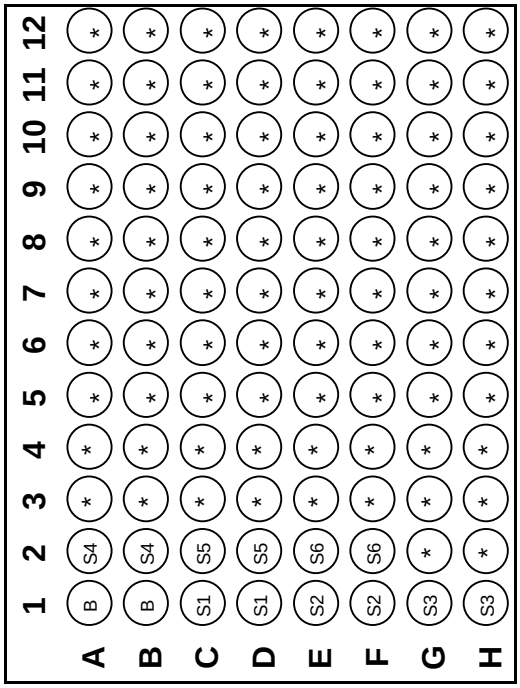
It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

### *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.




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). After final wash, invert and tap the plate strongly against paper towel.
- 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). After final wash, invert and tap the plate strongly against paper towel.

***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 (10 minutes at 20°C or up to 20 minutes is temperature is lower than 20°C). Avoid exposure to direct sunlight. It is rec ommended 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.

  Set of Standards are diluted 3x during reconstitution with the specified volume of EIA Buffer, and samples and QCs are all diluted 3x prior to analysis. Therefore, there is no need to take this dilution factor into account.
- 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*

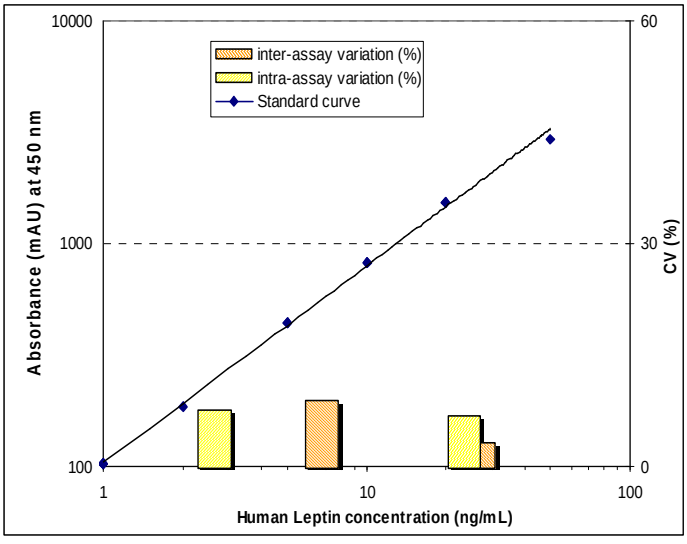
The following data are for demonstration purposes only. Your data may be different but still correct. These data were obtained using all reagents supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

| 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: $A_{blank} - 3*SD_{blank}$).

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

☞Precision:

- Intra-assay variation (n=8)

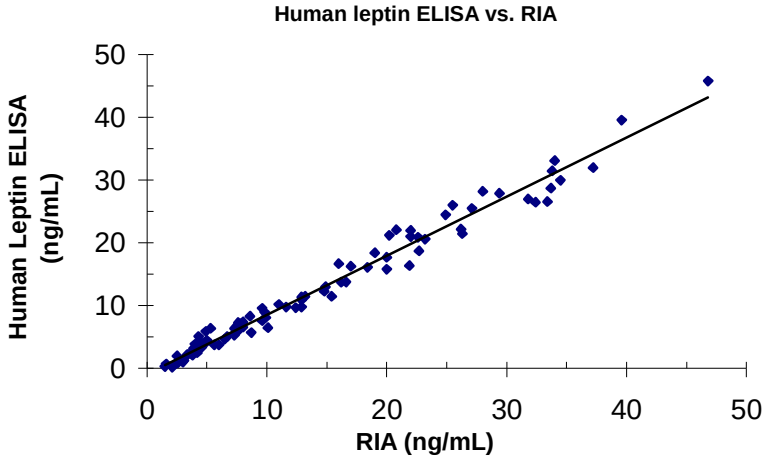
| Sample | Mean (ng/mL) | Standard Deviation (ng/mL) | CV (%) |
|---|---|---|---|
| 1 | 3.54 | 0.27 | 7.5 |
| 2 | 13.63 | 0.41 | 3.0 |
| 3 | 25.44 | 1.38 | 5.4 |
| 4 | 25.58 | 1.68 | 6.7 |

- 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 | 74.8 |
| | 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 | 95.2 |
| 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 60 36), fax (33 (0)1 39 30 62 99) or E-mail (bioreagent@bertinpharmacom), and be sure to indicate the lot number of the kit (see outside of the box).

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