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## MOUSE/RAT LEPTIN ENZYME IMMUNOASSAY KIT

catalogue # A05176

96 wells

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



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## MOUSE/RAT LEPTIN EIA KIT

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

This kit contains:

- ☞ A covered 96 well plate, pre-coated with a polyclonal anti-mouse Leptin antibody, ready to use
- ☞ One vial of biotin labelled anti-mouse Leptin antibody, ready to use
- ☞ One vial of Streptavidin tracer, ready to use
- ☞ One vial of mouse Leptin standard, lyophilised
- ☞ One vial of rat Leptin standard, lyophilised
- ☞ One vial of Substrate (TMB) solution, ready to use
- ☞ One vial of Stop solution (0.2 M H<sub>2</sub>SO<sub>4</sub>),
- ☞ Two vials of EIA Buffer,
- ☞ One vial of Biotin-Antibody buffer
- ☞ Two vials of mouse Quality Controls, lyophilised
- ☞ Two vials of rat Quality Controls, lyophilised
- ☞ 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 40 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 of reagent and dispense 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.

Wear gloves and laboratory coats are recommended when handling immunodiagnosics 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 is a protein hormone with important effects in metabolism and regulating body weight. It is a single-chain 16 kDa protein consisting of 146 amino acid residues and encoded by the obese (*ob*) gene. Leptin is expressed predominantly by adipocytes, small amounts of leptin are also secreted by cells in the epithelium of stomach and in the placenta. Leptin's effect on body weight is mediated through effects on hypothalamic centers, where leptin receptors are highly expressed. Leptin has a dual action, it decreases the appetite and increases energy consumption.

A mutations in the *ob* gene of leptin or in the gene of leptin receptor causes hyperphagia, reduced energy expenditure, and severe obesity in the *ob/ob* mice. *Ob* gene knockout mice are also characterized by several metabolic abnormalities including hyperglucocorticoidemia, hyperglycaemia, hyperinsulinemia and insulin

resistance. When *ob/ob* mice are treated with injections of leptin, they lose their excess fat and return to normal body weight.

Studies have shown that leptin appears to be a significant regulator of reproductive function. In addition, leptin is involved in bone metabolism and plays a significant role as an immunomodulator.

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 mouse Leptin. This antibody will bind any mouse/rat Leptin introduced in the wells (sample or standard).

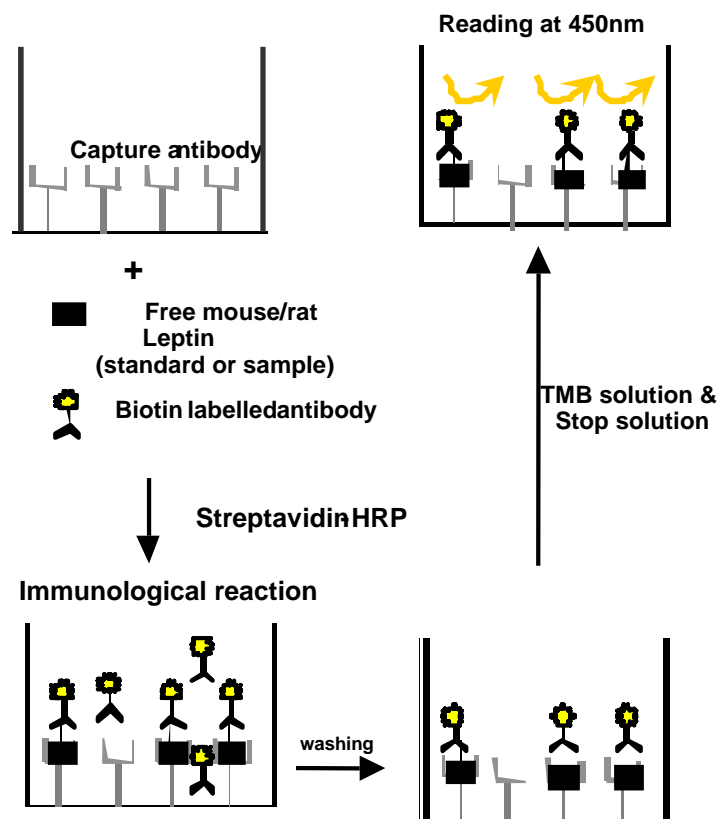
After one-hour incubation and a washing, biotin-labelled polyclonal anti-mouse Leptin antibody is added and incubated with captured Leptin during one hour.

After a thorough wash, streptavidin-horseradish peroxidase tracer is added and incubated for half an hour. This allows the two antibodies to form a sandwich by binding on different parts of the Leptin molecule.

The sandwich complex is immobilised on the plate so the excess reagents may be washed away. The concentration of the 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 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 (5 to 1000  $\mu$ L)
- ☞ Spectrophotometer plate reader (450 nm +/- 10 nm filter)
- ☞ Microtitration washer (or washbottles)
- ☞ Microplate shaker
- ☞ Multichannel pipette 100  $\mu$ L and disposable tips
- ☞ Distilled or deionised water
- ☞ Polypropylene tubes

## SAMPLE PREPARATION

This assay may be used to measure leptin in mouse/rat samples such as EDTA plasma and tissue 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 at -20°C, preferably -80°C.

### **SAMPLE PREPARATION**

Dilute samples 20 times in EIA buffer (i.e. 14  $\mu$ L sample + 266  $\mu$ L EIA buffer). If expected concentrations of Leptin are very low, dilute samples only 1/10 in EIA Buffer.

Do not store the diluted samples.

Avoid repeating freezing/thawing to maximum one cycle.

## REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Standard, Quality Control, Wash buffer, Biotin-labelled antibody.

Use mouse Leptin standard and mouse Leptin Quality Control to quantify Leptin concentration in mouse samples ; use rat Leptin standard and rat Leptin Quality Control to quantify leptin concentration in rat samples.

### ☞ Quality Controls

Reconstitute the vials with X  $\mu$ L of EIA buffer. The volume X is indicated on the vial of the corresponding Quality Control. Mix thoroughly by gentle inversion. Let stand for 15 minutes.  
The reconstituted QC has to be used immediately or to be frozen at -20°C.

### ☞ Leptin standards

Reconstitute Leptin standard with X  $\mu$ L of EIA buffer. The volume X is indicated on the vial of the corresponding standard. The concentration of the leptin in the stock solution (S1) is 4000 pg/mL.

Then prepare standards as follows:

Volume of Standards	Added volume of EIA buffer	Concentration of reconstituted standards
250 µL of S1	250 µL	S2 (2000 pg/mL)
250 µL of S2	250 µL	S3 (1000 pg/mL)
250 µL of S3	250 µL	S4 (500 pg/mL)
250 µL of S4	250 µL	S5 (250 pg/mL)
250 µL of S5	250 µL	S6 (125 pg/mL)
250 µL of S6	250 µL	S7 (62.5 pg/mL)

The reconstituted standards can be aliquoted and stored at -20°C until next use.

☞ Biotin-labelled antibody

Dilute the Biotin-labelled antibody with the Biotin-labelled buffer in needed quantity : 100 µL of Biotin-labelled antibody plus 900 µL Biotin-labelled buffer is sufficient for one strip. The diluted Biotin-labelled antibody is stable for 1 month at 2-8°C.

☞ Wash buffer

Reconstitute one vial of concentrated Wash buffer (100 mL, 10x) 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 content 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 performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

☞ Leptin standard:

Dispense 100 µL of each standard (S1 to S7: 62.5 to 4000 pg/mL) 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 100 µL of Quality Control and diluted samples in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

☞ EIA buffer:

Dispense in duplicate 100 µL in Blank (B) wells.



Enzyme Immunoassay Protocol (Volumes are in $\mu\text{L}$ )			
	Blank	Standard	Sample
EIA Buffer	100		
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 1 hour Wash the plate 3 times			
Biotin Labelled Leptin	-	100	100
Incubate the plate at room temperature for 1 hour Wash the plate 3 times			
Streptavidin-HRP	100	100	100
Incubate the plate at room temperature for 30 minutes Wash the plate 5 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10 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 logit-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. Take care to integrate the dilution factor of your samples (due notably to the minimal dilution for the assay 1/20).
- ↳ 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

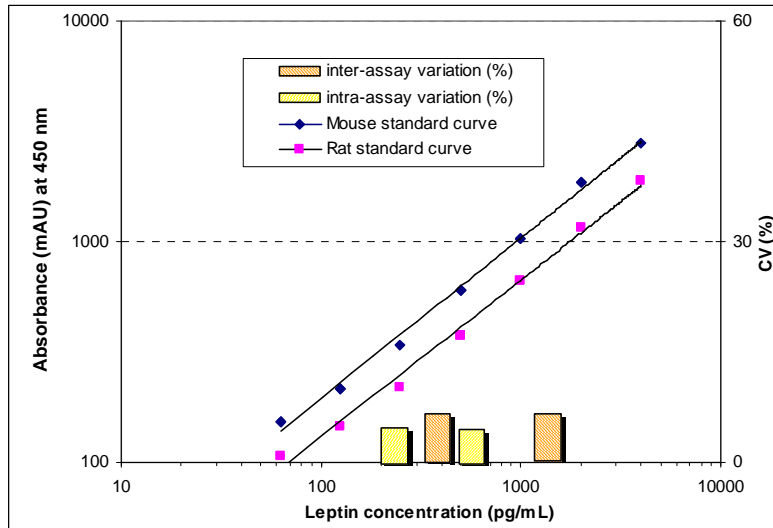
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Leptin	Mouse Leptin (mAU)	Rat Leptin (mAU)
Blank	71	44
Standard 4000 pg/mL	2779	1898
Standard 2000 pg/mL	1859	1162
Standard 1000 pg/mL	1034	666
Standard 500 pg/mL	602	376
Standard 250 pg/mL	338	219
Standard 125 pg/mL	216	145
Standard 62.5 pg/mL	154	106
QC	893	276

**ACCEPTABLE RANGE**

☞ QC samples: see label on the vials.

**MOUSE/RAT LEPTIN STANDARD CURVE**



**ASSAY VALIDATION AND CHARACTERISTICS**

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

☞ Cross-reactivity

- Mouse leptin: 100%
- Rat leptin: 100%

☞ Sensitivity

The limit of detection (defined as such a concentration of mouse/rat Leptin giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3 \cdot SD_{\text{blank}}$ ) is better than 50 pg/mL of sample. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞ Precision

- Intra-assay (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1 (mouse)	565.4	27.5	4.8
2 (mouse)	215.6	8.4	3.9
3 (rat)	245.7	13.9	5.7
4 (rat)	273.8	10.3	3.8

- Inter-assay (Run-to-Run, n=7)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1 (mouse)	303.6	18.4	6.0
2 (mouse)	481.0	30.7	6.4
3 (rat)	275.9	16.3	2.9
4 (rat)	243.9	17.1	7.0

☞ Recovery test

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1 (mouse)	367.9	-	-
	1113.5	1167.9	95.3
	777.9	767.9	101.3
	474.8	467.9	101.5
2 (mouse)	559.1	-	-
	1432.0	1359.1	105.4
	948.7	959.1	98.9
3 (rat)	681.2	659.1	103.3
	275.7	1075.7	97.2
	1045.8	675.7	100.0
4 (rat)	675.9	375.7	98.8
	371.3	186.1	98.2
	986.5	986.1	98.2
4 (rat)	605.7	586.1	103.3
	290.6	286.1	101.6
	986.5	986.1	98.2

☞ Dilution test

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	Recovery O/E (%)
1 (mouse)	-	34.77	-	-
	2x	16.78	17.39	96.5
	4x	8.35	8.69	96.0
	8x	4.06	4.35	93.3
2 (mouse)	-	23.44	-	-
	2x	11.69	11.72	99.7
	4x	5.85	5.86	99.8
	8x	2.77	2.93	94.6
3 (rat)	-	29.67	-	-
	2x	14.53	14.83	98.0
	4x	7.11	7.42	95.8
	8x	3.91	3.71	105.4
4 (rat)	-	40.78	-	-
	2x	19.86	20.39	97.4
	4x	9.84	10.19	96.6
	8x	5.23	5.10	102.7

## 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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