LEPTIN (mouse, rat)

A brand name of

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For laboratory research use only. Not for human or veterinary diagnostic use.

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Leptin (mouse/rat)
Enzyme Immunoassay kit
#A05176.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#A11176
Version: 0117

Table of contents

96 wells
Storage: +4°C
Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Leptin (mouse, rat) pre-coated Microtiter Plate	Blister with zip	A08176.1 ea	1	-
Leptin (mouse, rat) Biotin-labelled Antibody	Blue	A040176	1	Liquid
Streptavidin-HRP Tracer	Red	A22010	1	Liquid
Leptin (mouse) Standard	Yellow	A06176_M	2	Lyophilised
Leptin (rat) Standard	Yellow	A06176_R	2	Lyophilised
Leptin (mouse) QC	White	A10176_M	2	Lyophilised
Leptin (rat) QC	Green	A10176_R	2	Lyophilised
Biotin-labelled Antibody Dilution Buffer	Blue	A07014	1	Liquid
EIA Buffer	White	A07010	2	Liquid
Concentrated Wash Buffer	White bottle	A17072	1	Liquid
Substrate Solution (TMB)	Black	A09010	1	Liquid
Stop Solution	Yellow	A22000	1	Liquid
Technical Booklet	-	A11176.1 ea	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 40 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard and one of Quality Control.

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

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

Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

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.

Background

Leptin *[1 - 9]*

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 mutation 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.

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a double-antibody sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to Leptin (mouse). This antibody will bind any Leptin (mouse, rat) introduced in the wells (Sample, QC 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. The two antibodies form a sandwich by binding on different parts of the Leptin molecule.

After a thorough wash, Streptavidin-horseradish peroxidase Tracer is added and incubated for half an hour.

The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP.

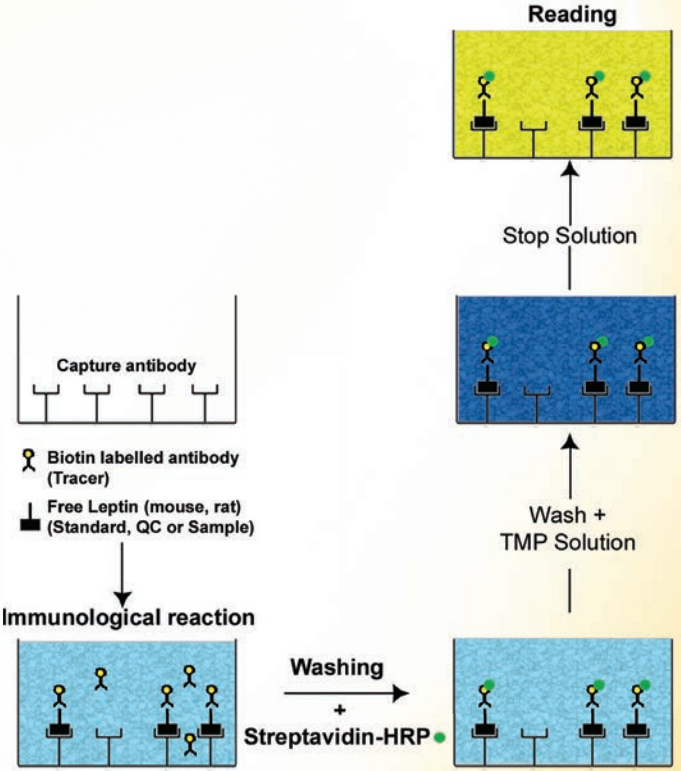
The concentration of the Leptin is then determined by measuring the enzymatic activity of the HRP using TMB solution.

The HRP tracer acts on TMB Reagent to form a yellow compound.

The reaction is stopped by addition of sulfuric acid solution.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of 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 1000 µL)
- Spectrophotometer plate reader (450 +/- 10 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water #A07001.1L
- Polypropylene tubes

Water used to prepare all EIA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Do not use distilled water, HPLC-grade water or sterile water.
UltraPure water may be purchased from Bertin Pharma: item #A07001.1L.

Sample collection and preparation

This assay may be used to measure Leptin in mouse and rat samples such as serum, plasma (EDTA, citrate, heparin) and tissue culture supernatant.

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. Avoid repeating freeze/thaw cycles.

Sample preparation

Dilute samples 20 times in EIA buffer (i.e. 14 µL sample + 266 µL EIA buffer for duplicates) and mix well (not to foam). Vortex is recommended.

If expected concentrations of Leptin are very low, dilute samples only 1/3 and/or 1/10 in EIA Buffer.

Do not store the diluted samples.

Reagent preparation

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, Wash buffer and Biotin-labelled antibody. Opened reagents are stable 3 months at +4°C.

Substrate Solution (TMB) should remain colourless until added to the plate. Keep it protected from the light.

Use Leptin (mouse) Standard and Quality Control to quantify Leptin concentration in mouse samples.

Use Leptin (rat) Standard and Quality Control to quantify Leptin concentration in rat samples.

Leptin (mouse or rat) Standard

Reconstitute one Leptin (mouse or rat) Standard vial #A06176_M or #A06176_R with X mL of EIA Buffer. The volume X is indicated on the vial of the corresponding standard. Allow it to stand 15 minutes with occasional gentle shaking (not to foam) until completely dissolved.

The concentration of this first standard (S1) is 4000 pg/mL. Prepare five polypropylene tubes for the five other standards (S2 to S6). Then prepare the standard concentrations by serial dilutions as follow:

Standard	Volume of Standard	Volume of EIA Buffer	Standard concentration (pg / mL)
S2	250 µL of S1	250 µL	2000
S3	250 µL of S2	250 µL	1000
S4	200 µL of S3	300 µL	400
S5	250 µL of S4	250 µL	200
S6	250 µL of S5	250 µL	100

Stability at 4°C: within the day.

The reconstituted standards can be aliquoted and stored at -20°C for 3 months.

▷ Leptin (mouse or rat) Quality Control

Reconstitute one Leptin (mouse or rat) Quality Control vial #A10176_M or #A10176_R with X mL of EIA Buffer. The volume X is indicated on the vial of the corresponding Quality Control. Allow it to stand 15 minutes with occasional gentle shaking (not to foam) until completely dissolved.

The reconstituted Quality Control (QC) must be used immediately or aliquoted and stored at -20°C for 3 months.

▷ Biotin-labelled Antibody

Dilute 10 times the Biotin-labelled Antibody with the Biotin-labelled Antibody Dilution Buffer in needed quantity: 100 µL of Antibody + 900 µL of Buffer is sufficient for one strip.
Stability at +4°C: 1 month.

Wash Buffer

Dilute 100 mL of concentrated Wash Buffer #A17000 with 900 mL of UltraPure water.
Stability at +4°C: 1 month.

Assay procedure

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

Plate preparation

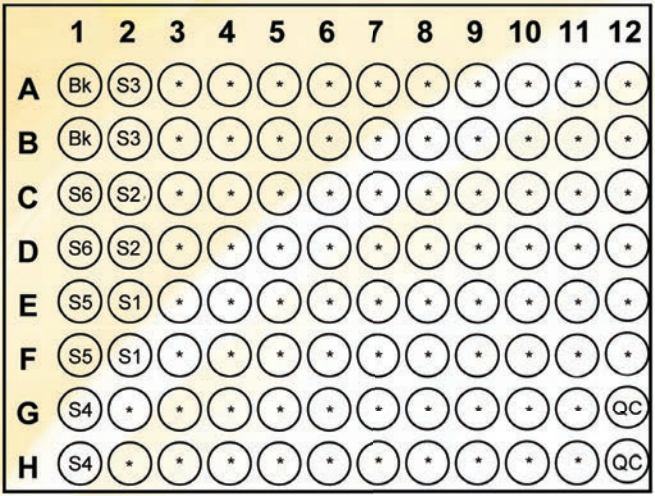
Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay. Put unused strips back in the zip lock bag with the dessicant pocket and properly close it.
Stability at +4°C: 3 months.

Rinse each well 5 times with Wash Buffer (300 µL/well).

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

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.



Bk: Blank
*: Samples
S1-S6: Standards 1-6
QC: Quality Control

▷ Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet all the 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.

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

> **Leptin (mouse or rat) Quality Control and Samples**
Dispense 100 µL in duplicate to appropriate wells.

> **EIA Buffer**
Dispense 100 µL in duplicate to the Blank (BK) wells.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate 1 hour at room temperature, shaking at 300 rpm on an orbital microplate shaker.

▷ Washing the plate

Rinse each well 3 times with Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

▷ Pipetting the reagents

Dispense 100 µL of Biotin-labelled Antibody to each well, except Blank (Bk) wells.

Incubating the plate

Cover the plate with the cover sheet and incubate 1 hour at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

Dispense 100 µL of Streptavidin-HRP Tracer to each well, except Blank (Bk) wells.

Incubating the plate

Cover the plate with the cover sheet and incubate 30 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with Wash Buffer (300 µL/well). Just before distributing reagents, 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

- Add 100µL of Substrate Solution to each well. Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil.

- Incubate the plate in the dark during 10 minutes at room temperature. Do not shake the plate during the incubation. (if the reaction temperature is below 20°C, the incubation time may be extended up to 20 minutes).

- Stop the colour development by adding 100 µL of Stop Solution.

- Read the absorbance at 450 nm within 5 minutes following Stop Solution addition (yellow color).

Note:
If some sample(s) and standard(s) have absorbances above the upper limit of your microplate reader, 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 reading for samples that were “in range” at 450 nm.

Enzyme Immunoassay Protocol (volumes are in µL)			
Volume / Wells	Blank	Standard	Sample or QC
EIA Buffer	100	-	-
Standard	-	100	-
Sample or QC	-	-	100
Cover plate, incubate 1 hour at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper			
Biotin-labelled Antibody	100		
Cover plate, incubate 1 hour at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper			
Streptavidin-HRP Tracer	100		
Cover plate, incubate 30 minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper			
Substrate Solution(TMP)	100		
Incubate 10 minutes the plate in the dark without agitation			
Stop Solution	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 now.

> Calculate the average absorbance for each standard, sample and QC.

> For each standard, plot the absorbance on *y* axis versus the concentration on *x* axis. 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.
Take care to integrate the dilution factor of your samples (due notably to the minimal dilution for the assay 1/20).

> Samples with a concentration greater than 4000 pg/mL should be re-assayed after dilution in EIA Buffer.

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

Acceptable range

> QC Samples: +/- 30% of the expected concentration (see the label of QC vial).

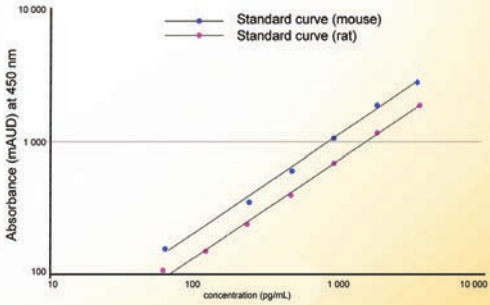
Typical results

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

These data were obtained using all reagents as supplied in this kit under the following conditions: 10 minutes developing at room temperature, reading at 450 nm. A 4 parameter logistic fitting was used to determine the concentrations.

Standard	Leptin concentration (pg / mL)	Leptin (mouse) (A.U.)	Leptin (rat) (A.U.)
S1	4000	3.194	2.549
S2	2000	2.124	1.805
S3	1000	1.215	0.968
S4	400	0.501	0.396
S5	200	0.262	0.203
S6	100	0.096	0.075
Blank	0	0.002	0.001
QC	-	1.148	0.819

Typical Leptin standard curve



Assay validation and characteristics

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

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography ***[10, 11]***.

- The **Limit of detection (LOD)** (defined as such a concentration of Leptin giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: $A_{blank} + 3*SD_{blank}$) is 30 pg/mL for Leptin (mouse) and 50 pg/mL for Leptin (rat).
  The EIA Buffer was pipetted into blank wells, and the microtiter plate was blanked on air.

- **Limit of quantification** in EIA Buffer: 4000 pg/mL

> Cross-reactivity

Leptin (mouse)	Yes
Leptin (rat)	Yes
Leptin (human)	Yes
Leptin (Bovine)	No
Leptin (Cat)	No
Leptin (Dog)	No
Leptin (Goat)	No
Leptin (Hamster)	No
Leptin (Horse)	No
Leptin (Monkey)	No
Leptin (Pig)	No
Leptin (rabbit)	No

> Intra-assay variation (n = 8) in EIA Buffer

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1 (mouse)	12.31	0.25	2.0
2 (mouse)	31.48	0.91	2.9
3 (rat)	9.74	0.18	1.8
4 (rat)	39.96	0.75	1.9

> Inter-assay variation (n = 6) in EIA Buffer

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1 (mouse)	21.32	0.48	2.3
2 (rat)	17.13	0.76	4.4

> Recovery test

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1 (mouse)	12.02	-	-
	15.19	16.02	94.8
	18.46	20.02	92.2
	25.90	28.02	92.4
2 (mouse)	19.56	-	-
	21.86	23.56	92.8
	24.04	27.56	87.2
	32.03	35.56	90.8
3 (rat)	9.32	-	-
	13.33	13.32	100.1
	16.29	17.32	94.1
	25.47	25.35	100.6
4 (rat)	19.61	-	-
	22.41	23.61	94.6
	25.25	27.61	95.1
	34.83	35.6	97.8

> Dilution test

Sample	Dilution	Observed (ng/mL)	Expected (ng/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	93.4
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	105.5
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

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 with substrate solution should be reduced before adding Stop Solution),
  - 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 Pharma 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 (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Marketing Department by phone (+33 (0)139 306 260) or E-mail (marketing@bertinpharma.com).

Bibliography

1. Gruver AL., Sempowski GD.
Cytokines, leptin, and stress-induced thymic atrophy.
J Leukoc Biol. (2008) May

2. Cherhab FF., Mounzih K., Lu R. *et al.*
Early onset of reproductive function in normal mice treated with Leptin.
Science 275, 88 (1997).

3. Clement K., Vaisse C., Lahlou . *et al.*
A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction.
Nature 329, 398 (1998).

4. Considine R.V., Sinha M.K., Heiman M.L. *et al.*
Serum immunoreactive leptin concentrations in normal-weight and obese humans.
N. Engl. J. Med. 334, 292-295 (1996)

5. Friedman JM., Halaas JL.
Leptin and regulation of body weight inmammals.
Nature 395, 763 (1998).

6. Halaas J.L., Gajiwala K.S., Maffei M.. *et al.*
Weight-reducing effects of the plasma protein encoded by the obese gene.
Science 269, 543-546 (1995)

7. Montague CT., Faroozi IS., Witehead JP. *et al.*
Congenital leptin deficiency deficiency is associated with serve early-onset obesity in humans.
Nature 387, 903 (1997)

8. Pelleymounter M.A., Cullen M.J., Baker M.B. *et al.*
Effects of the obese gene product on body weight regulation in ob/ob mice.
Science 269, 540-543 (1995)

9. Zhang Y., Proenca R., Maffei M.. *et al.*
Positional cloning of the mouse obese gene and its human homologue.
Nature 372, 425-432 (1994)

10. Valentin MA., Ma S., Zhao A. *et al*.
Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies.
J Pharm Biomed Anal. (2011) 55(5) : 869-877

11. European Medicines Agency
Guideline on bioanalytical method validation, 21 July 2011

Additional readings

List of publications quoting the use of this kit

12. Mashmoul M., Azlan A, Mohtarrudin N. *et al.*
Saffron Extract and Crocin Reduced Biomarkers Associated with Obesity in Rats Fed a High-Fat Diet.
Mal J Nutr 23(1) : 117 - 127, 2017

13. Noaman I.M.,
The Effect of Serotonin on Leptin and Grelin Hormones Concentrations in Female Rats.
Kufa Journal For Veterinary Medical Sciences Vol. (6) No. (2) 2015

14. Hoile SP., Grenfell LM., Hanson MA. *et al.*
Fat and carbohydrate intake over three generations modify growth, metabolism and cardiovascular phenotype in female mice in an age-related manner.
PLoS One. 2015 Aug 12 ;10(8) : e0134664

15. Lefils J., Géloën A., Vidal H. *et al.*
Dietary DHA : time course of tissue uptake and effects on cytokine secretion in mice.
Br J Nutr. 2010 Nov ;104(9) :1304-12

16. Aminzadeh MA., Pahl MV., Barton CH. *et al.*
Human uraemic plasma stimulates release of leptin and uptake of tumour necrosis factor-alpha in visceral adipocytes.
Nephrol Dial Transplant. 2009 Dec ;24(12) :3626-31

17. Pye KM., Wakefield AP., Aukema HM. *et al.*
A high mixed protein diet reduces body fat without altering the mechanical properties of bone in female rats.
J Nutr. 2009 Nov ;139(11) :2099-105

18. Terao S., Yilmaz G., Stokes KY. *et al.*
Inflammatory and injury responses to ischemic stroke in obese mice.
Stroke. 2008 Mar ;39(3) :943-50

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Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and biochemicals thanks to its innovative work in research and development.
Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

Bertin Pharma is active worldwide either with direct sales or through our qualified and trained international distribution network from the United States to Japan.

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