LEPTIN (mouse, rat)

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

Bertin Bioreagent also markets pre-analytical products, EIA kits, antibodies & biochemicals for:

- **Inflammation**
- **Oxidative injury**
- **Endocrinology**
- **Diabetes**
- **Obesity**
- **Hypertension**
- **Pain**
- **Prion diseases**

Do not hesitate to contact our after-sales services for further information at tech@bertin-bioreagent.com

Leptin (mouse, rat)

ELISA kit

#A05176.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11176

Version: 0123

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) precoated 96-well Strip Plate	Blister with zip	A08176.1 ea	1	-
Streptavidin-HRP Tracer	Red	A22010	1	Liquid
Leptin (mouse, rat) Biotin-labelled	Blue	A040176	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
ELISA 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 (TMB) 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 and decreases its efficiency.

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 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 (HRP) Tracer is added and incubated for half an hour.

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

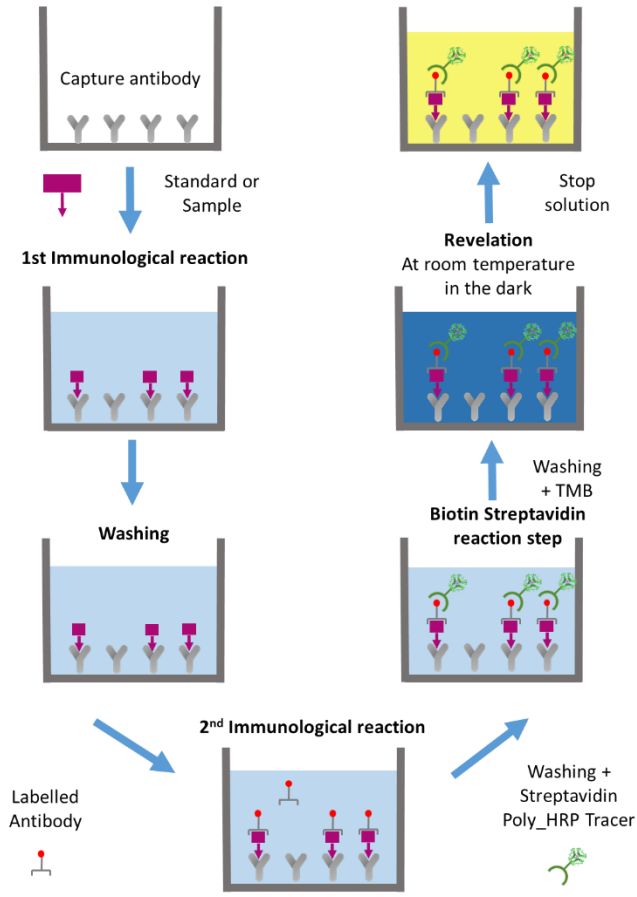
The concentration of the Leptin is then determined by measuring the enzymatic activity of the HRP using substrate solution (TMB).

The HRP tracer acts on the substrate solution to form a yellow compound.

The reaction is stopped by the addition of an acidic solution.

The intensity of the color, 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:



Assay characteristics

- ***Validated for***
  - Human species
  - Mouse species

- ***Limit of detection (LOD):*** 30 pg/ml for mouse leptin and 50 pg/ml for rat leptin. LOD is defined as the concentration of Leptin giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$).
  *EIA buffer is pipetted into blank wells.

- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
Bovine Leptin	No
Cat Leptin	No
Dog Leptin	No
Goat Leptin	No
Hamster Leptin	No
Horse Leptin	No
Human Leptin	Yes
Monkey Leptin	No
Mouse Leptin	Yes
Pig Leptin	No

Rabbit Leptin	No
Rat Leptin	Yes

> *Precision*

- Intra-assay (Within-Run) (n=8)

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

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

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

> *Recovery test (serum samples)*

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

1 rat	9.32 13.33 16.29 25.47	- 13.32 17.32 25.32	- 100.1 94.1 100.6
2 rat	19.61 22.41 25.25 34.83	- 23.61 27.61 35.61	- 94.9 95.1 97.8

> *Dilution test (serum samples)*

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1 mouse	- 1:2 1:4 1:8	34.77 16.78 8.35 4.06	- 17.39 8.69 4.35	- 96.5 96.0 93.4
2 mouse	- 1:2 1:4 1:8	23.44 11.69 5.85 2.77	- 11.72 5.86 2.93	- 99.7 99.8 94.6
3 rat	- 1:2 1:4 1:8	29.67 14.53 7.11 3.91	- 14.83 7.42 3.71	- 98.0 95.8 105.5
4 rat	- 1:2 1:4 1:8	40.78 19.86 9.84 5.23	- 20.39 10.19 5.10	- 97.4 96.6 102.7

>

Materials and equipment required

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

For the assay:

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Buffer (Dilution Buffer)
- Precision micropipettes and disposable tips (5-1000 µL)
- Multichannel pipette and disposable tips of 100 µL
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker (300 rpm)
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm).

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

- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of animal origin. These materials should be handled as potentially infectious.
- 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 contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solution may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

Technical hints

- Reagents with different lot numbers should not be

mixed.

- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from the light.
- Stop Solution should remain colorless until added to the plate. The color developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Sample preparation

No prior extraction procedure is necessary.

To measure mouse or rat Leptin, dilute samples at 1:20 in EIA Buffer (i.e. 14 µL sample + 266 µL EIA Buffer), 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

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

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 µL of EIA 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 (mouse or rat) in the stock solution (S1) is 4000 pg/mL.

Prepare five polypropylene tubes (for the 5 other standards) and add EIA 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 EIA Buffer	Standard concentration (pg/mL)
S1	-	X (see label)	4000
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

Prepared standards are ready to use, do not dilute them.

Stability: Reconstituted Master Standard (4000 pg/ml) must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted Standard solutions.

Leptin (mouse or rat) Quality Control

Reconstitute one Leptin (mouse or rat) QC vial #A10176_M or #A10176_R with X 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. Avoid repeated freeze/thaw cycles.

Biotin-labelled Antibody

Prepare the working Biotin-labelled Antibody solution by adding 1 part of Biotin-labelled Antibody Concentrate (10x) with 9 parts of Biotin-labelled Antibody Dilution Buffer. Example: 100 µL of Biotin-labelled Antibody +900 µL of Biotin-labelled Antibody Dilution Buffer.

Stability of Biotin-labelled Antibody Concentrate (10x) *at +4°C: 3 months.*

Do not store the diluted Biotin-labelled Antibody solution.

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.

Assay procedure

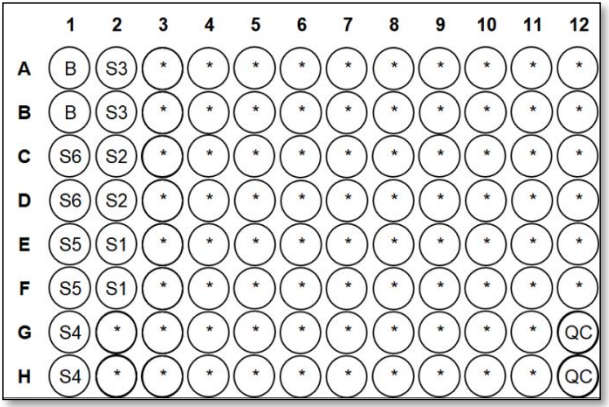
It is recommended to perform the assays in duplicate following the instructions hereafter.

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.



B: Blank
QC: Quality Control
S1-S6: Standards 1-8
*: Samples

Pipetting the reagents

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

Use different tips to pipet the buffers, standards, samples, antibody 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 duplicate to the Blank (B) wells.

- ***Leptin (mouse or rat) Standard***

Dispense 100 µL of each of the six standards (S6 to S1) in duplicate in 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.

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 (350 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 solution to each well.

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 (350 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.

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 (350 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 100 µL of Substrate Solution to each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.

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

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

- 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 readings for samples that were "in range" at 450 nm.***

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
Volume / Wells	Blank	Standard	Sample or QC
ELISA 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 350 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 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Streptavidin-HRP Tracer	100		
Cover plate, incubate **30 min** 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 (TMP)	100		
Incubate **10 min** the plate in the dark without agitation			
Stop Solution	100		
Read the plate at 450 nm			

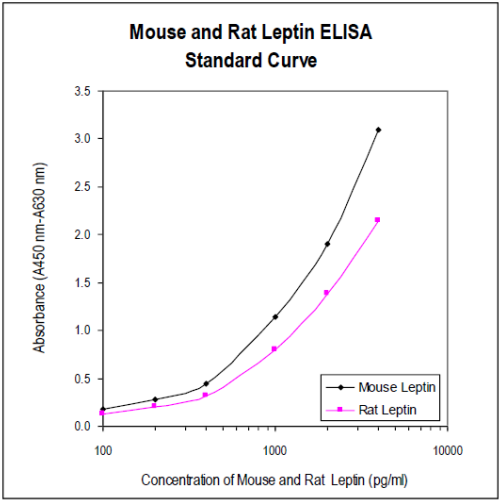
Data analysis

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 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 EIA 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.



Typical standard curve for Mouse and Rat Leptin ELISA

Acceptable range

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

Troubleshooting

Weak signal in all wells:

- Omission of a reagent or a step,
- Improper preparation or storage of a reagent,
- Assay performed before reagents were allowed to come to room temperature,
- Improper wavelength when reading absorbance.

High signal and background in all wells:

- Improper or inadequate washing,
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution,
- Incubation temperature over 30 °C.

High Coefficient of Variation (CV):

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples.

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.

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 in mammals. *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 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. Lefi ls 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., Wakefi eld 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*

1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

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 Homogenization and Digital Imaging.

Our products are available worldwide through us directly or via our distributor network. Our sales team is active on all continents and will be delighted to answer all your commercial questions.

Should you need help with a product, you can contact our technical support by emailing to tech@bertin-bioreagent.com
Should you need help with an order, you can contact our customer service by emailing to order@bertin-bioreagent.com

Please visit our websites:
Bertin-instruments.com
Bertin-Bioreagent.com
Bertin-Corp.com

CONTACT US

Bertin Technologies
10 bis Avenue Ampère
Parc d'Activités du Pas du Lac
78180 Montigny-le-Bretonneux
FRANCE

+33 (0)139 306 036

tech@bertin-bioreagent.com

EU webstore: Bertin-bioreagent.com
US webstore: Bertin-corp.com