PROLACTIN (rat)

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

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- **Prion diseases**

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

Prolactin (rat)

ELISA kit

#A05101.96 wells

For research laboratory use only
Not for human diagnostic use

This assay was developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11101
Version: 0120

Table of contents

96 wells
Storage: -20°C
Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Qty per kit	Form
Mouse anti-Rabbit precoated 96-well Strip Plate	Blister with zip	A08100.1 ea	1	-
Prolactin (rat) Tracer	Green	A04101.100 dtn	1	Lyophilised
Prolactin (rat) Antiserum	Red	A03101.100 dtn	1	Lyophilised
Prolactin (rat) Standard	Blue with red septum	A06101.1 ea	2	Lyophilised
Prolactin (rat) Quality Control	Green with red septum	A10101.1 ea	2	Lyophilised
ELISA Buffer	Blue	A07000.1 ea	1	Lyophilised
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween® 20	Transparent	A12000.1 ea	1	Liquid
Ellman's reagent 50	Black with red septum	A09000_50.100 dtn	2	Lyophilised
Technical booklet	-	A1110101 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 35 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 where kit reagents are handled
- Avoid splashing

The QC samples provided in this kit have been prepared by diluting rat plasma (Sprague Dawley rat) in ELISA buffer. A sanitary control has been completed on Sprague Dawley rats following the Felasa Health Monitoring Recommendations. However, handle the CQ samples as a possible source of infection.

The total amount of reagents contain less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

Wearing lab gloves, laboratory coat and eye protection 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

Acetylcholinesterase AChE® Technology

Acetylcholinesterase (AChE®), the enzymatic label for ELISA, has the fastest turnover rate of any enzymatic label. This specific AChE® is extracted from the electric organ of the electric eel, *Electrophorus electricus*, and it is capable of providing a rapid catalytic turnover during the generation of the electrochemical discharges. The use of AChE® as enzymatic label for ELISA is patented by the French academic research Institute CEA [1–3], and Bertin Bioreagent has expertise to develop and produce ELISA kits using this technology.

AChE® assays are revealed with Ellman's reagent, which contains acetylthiocholine as a substrate. The final product of the enzymatic reaction (5-thio-2-nitrobenzoic acid), is bright yellow in color and can be read at 405-414 nm using a spectrophotometer. AChE® offers several advantages over other commonly used enzymes used in ELISAs:

- **Kinetic superiority and high sensitivity:** AChE® shows true first-order kinetics with a turnover of 64,000 sec^{-1}. That is nearly 3 times faster than Horse Radish Peroxidase (HRP) or alkaline

phosphatase. AChE® provides greater sensitivity than other labeling enzymes.

- **Low background**: Non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent. Thus, AChE® ensures a very low background and an increased signal/noise ratio compared to other substrate of enzymes that are inherently unstable.

- **Wide dynamic range:** AChE® is a stable enzyme and its activity remains constant for many hours, unlike other enzymes, its substrate is not suicidal. This permits simultaneous assays of high diluted and very concentrated samples.
- **Versatility:** AChE® is a completely stable enzyme, unlike peroxidase which is suicidal. Thus, if a plate is accidentally dropped after dispatch of the AChE® substrate (Ellman's reagent), one only needs to wash the plate, add fresh Ellman's reagent and proceed with a new development. Otherwise, the plate can be stored at +4°C while waiting for technical advice from the Bioreagent Department.

Prolactin

Prolactin (PRL) is a pituitary hormone whose molecular weight is approximately 23000 Daltons [4].
It is a single polypeptide chain composed of about 200 amino acid residues with three disulphide bonds.

In mammals, Prolactin has been claimed to exert a wide range of different physiological effects. These include stimulation of mammary gland development and lactation, hair maturation, synergism with androgen in male sex accessory growth and maintenance and secretion of corpus

luteum. PRL is predominantly under inhibitory control by the hypothalamus.

Stimulation of Prolactin release can be mediated by dopamine and thyrotrophin-releasing hormone (TRH).

Principle of the assay

The enzymatic immunoassay (ELISA) is based on the competition between unlabelled (free) rat Prolactin (standards / QC / samples) and acetylcholinesterase (AChE) linked to rat Prolactin (Tracer) for limited specific rabbit anti-rat Prolactin antiserum sites.

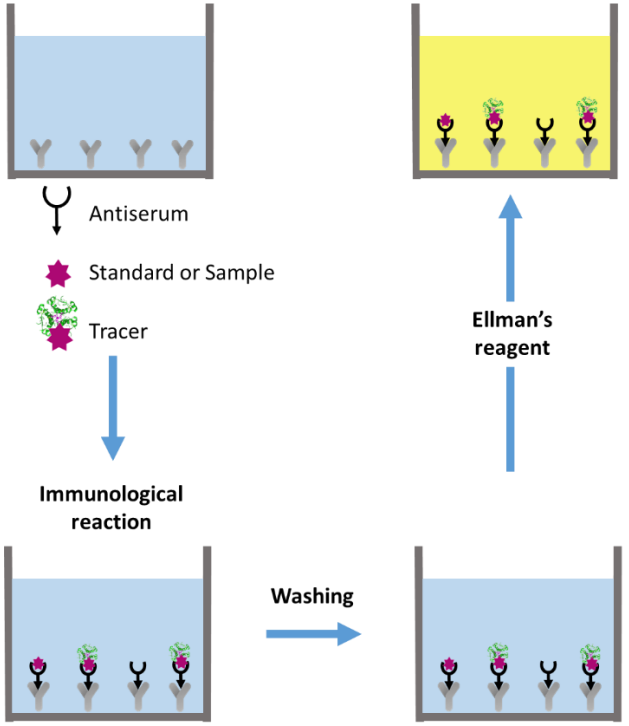
The complex rabbit antiserum – rat Prolactin (free Prolactin or Tracer) binds to the mouse monoclonal anti-rabbit antibody coated in the well.

The plate is washed to remove any unbound reagent, and Ellman's reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

The AChE tracer acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm.

The intensity of the colour, determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat Prolactin present in the well during the immunological incubation.

The principle of the assay is summarised below:



Assay characteristics

The Enzyme Immunometric assay of Prolactin (rat) has been validated by Duhau et al. for its use in rat plasma [5] .

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography [6,7] .

- **Limit of detection** (LOD) calculated as the concentration of Prolactin corresponding to the B0 average minus three standard deviations: 0.2ng/mL
- **Limit of quantification**: 1 ng/mL
- Quality control (QC) samples **intra & inter-assay variation**: established by measuring each QC five times per assay and in six different assays (ie. 30 assays per QC):

	Plasma QC (1ng/mL)	Plasma QC (4.6ng/mL)	Plasma QC (11.5ng/mL)
Mean value	1.15	4.60	11.7
Number of values	30	30	30
Intra-assay coefficient of variation (%)	11.5	10.6	10.0
Inter-assay coefficient of variation (%)	13.4	14.8	19.3
Recovery (%) +/- confidence intervalle	112.5 ± 5.4	89.5 ± 9.5	102.8 ± 7.5

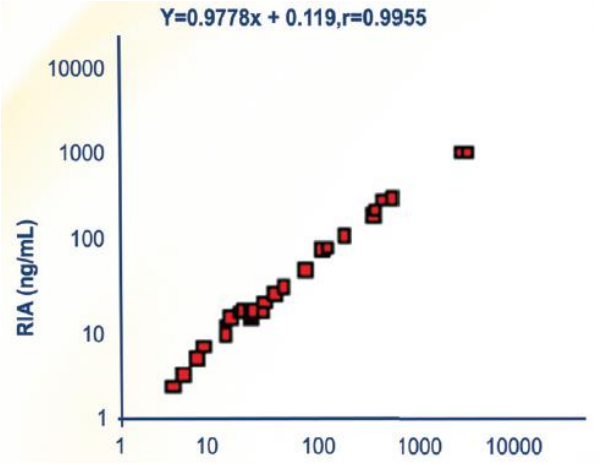
> ***Cross-reactivity :***

with rat LH, rat GH & rat TSH: <1%.

> ***Accuracy***

Prolactin added	Prolactin measured	Recovery (ng/mL)	Recovery (%)
0 ng/mL	26 ng/mL	-	-
10 ng/mL	37 ng/mL	11 ng/mL	110%
20 ng/mL	51 ng/mL	25 ng/mL	125%
40 ng/mL	75 ng/mL	49 ng/mL	123%
60 ng/mL	95 ng/mL	69 ng/mL	115%

> ***Comparison with RIA*** *on 26 rat plasma samples*



> ***Rat plasma level ranging***

- Male: 8 to 33 ng/mL (n=8)
- Female: 43 to 977 ng/mL (n=18)

Materials and equipment required

In addition to standard laboratory equipment, the following materials are required:

- Precision micropipettes (20 to 1000 µL)
- Multichannel pipette and disposable tips 30-300 µL
- Spectrophotometer plate reader (405 or 415 nm filter)
- Microplate washer (or washbottles)
- Microplate shaker
- Magnetic stirring bar
- UltraPure water #A07001
- Polypropylene tubes

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Othrewise, organic contamination can significantly affect the enzymatic activity of the tracer Acetylcholinesterase (AChE).
Do not use distilled water, HPLC-grade water or sterile water.

- UltraPure water may be purchased from Bertin Bioreagent (item #A07001.1L)

Sample collection and preparation

This assay has been validated to measure Prolactin in rat plasma or serum sample.

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.

Blood sample collection

Blood samples are collected in tubes containing lithium heparin, ADTA, potassium oxalate or sodium citrate for plasma collection.

The samples are centrifuged at 1,600 g for 20 minutes. Plasma are collected and kept at -20°C until assay.

Blood sample preparation

Samples are thawed, vortexed and centrifuged at 1,600 g for 20 minutes on the assay day, to eliminate fibrin.

No prior extraction procedure is necessary to measure Prolactin in plasma samples.

Other kind of samples

Prolactin can be assayed with this kit in cell culture supernatants without prior extraction. Please refer to literature for additional information [8,9] .

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 35 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard, one of quality Control and one of Ellman's Reagent.

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

ELISA Buffer

Reconstitute the ELISA Buffer #A07000 with 50 mL of UltraPure water. Allow buffer to stand for 5 minutes or until it is completely dissolved. Mix buffer thoroughly by gentle inversion.
Stability at 4°C: 1 month.

Prolactin (rat) Standard

Reconstitute one Standard vial #A06101 with 1mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of this first standard **S1** is 50 ng/mL.

Prepare seven propylene tubes for the other standards and add 500 µL of ELISA Buffer into each tube. Then prepare the standards by serial dilutions as follows:

Standard	Volume of Standard	Volume of ELISA Buffer	Standard concentration ng/mL
S1	-	-	50.00
S2	500 µL of S1	500 µL	25.00
S3	500 µL of S2	500 µL	12.5
S4	500 µL of S3	500 µL	6.25
S5	500 µL of S4	500 µL	3.13
S6	500 µL of S5	500 µL	1.56
S7	500 µL of S6	500 µL	0.78
S8	500 µL of S7	500 µL	0.39

Stability at 4°C: 1 week

Prolactin (rat) Quality Control

Reconstitute one vial #A10101 with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

Stability at 4°C: 1 week

Prolactin (rat) Tracer

Reconstitute the vial #A04101 with 5 mL of ELISA Buffer. Allow it to stand 5 minutes until completely

dissolved and then mix thoroughly by gentle inversion.

Stability at +4°C: 1 month

Prolactin (rat) Antiserum

Reconstitute the vial #A03101 with 5 mL of ELISA Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

Stability at +4°C: 1 week

Wash Buffer

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL of UltraPure water. Add 400 µL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content.

Stability a +4°C: 1 month

Ellman's Reagent

5 minutes before use (development of the plate), reconstitute one vial of Ellman's Reagent #A09000_50 with 50 mL of UltraPure water. The tube content should be thoroughly mixed.

Stability at 4°C and in the dark: 24 hours

Assay procedure

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

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate pouch and select enough strips for your assay and place the unused strips back in the pouch, store at +4°C for 1 month maximum.

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

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and blot the last drops by tapping it on paper towels.

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	Bk	S8	S4	*	*	*	*	*	*	*	*	*
B	Bk	S8	S4	*	*	*	*	*	*	*	*	*
C	NSB	S7	S3	*	*	*	*	*	*	*	*	*
D	NSB	S7	S3	*	*	*	*	*	*	*	*	*
E	NSB	S6	S2	*	*	*	*	*	*	*	*	*
F	B0	S6	S2	*	*	*	*	*	*	*	*	*
G	B0	S5	S1	*	*	*	*	*	*	*	*	QC
H	B0	S5	S1	*	*	*	*	*	*	*	*	QC

Bk : Blank
NSB : Non Specific Binding
QC : Quality Control
B0 : Maximum Binding
S1-S8 : Standards 1-8
* : Samples

Pipetting the reagents

Samples and reagents must reach room temperature prior performing the assay.

Use different tips to pipette the buffer, standard, sample, tracer, antiserum 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 to NSB wells and 50 µL to B0 wells.

> *Prolactin (rat) Standard*

Dispense 50 µL of each of the eight standards **S8** to **S1** in duplicate to appropriate wells.

Start with the lowest concentration standard **S8** and equilibrate the tip in the next higher standard before pipetting.

> *Prolactin (rat) Quality Control and Samples*

Dispense 50 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in ELISA Buffer.

> *Prolactin (rat) Tracer*

Dispense 50 µL to each well, **except** Blank (Bk) wells.

> *Prolactin (rat) Antiserum*

Dispense 50 µL to each well **except** Blank (Bk) wells and Non Specific Binding (NSB) wells.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate 16-20 hours at room temperature.

Developing and reading the plate

- Reconstitute Ellman's reagent as mentioned in the Reagent preparation section.

- Empty the plate by turning it over. Rinse each well five times with 300 µL Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

- Add 200µL of Ellman's reagent to each well. Cover the plate with an aluminium foil sheet and incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker.

- Gently wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.

- Read the plate at a wavelength between 405 and 414nm (yellow colour).

After addition of Ellman's reagent, the absorbance has to be checked periodically (every 30 minutes) until the maximum absorbance (B0 wells) has reached a minimum of 0.200 A.U. (blank subtracted).

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)						
	Blank	NSB	B0	Standard	QC	Sample
ELISA Buffer	-	100	50	-	-	-
Standard	-	-	-	50	-	-
QC	-	-	-	-	50	-
Sample	-	-	-	-	-	50
Tracer	-	50	50	50	50	50
Antiserum	-	-	50	50	50	50
Cover plate, incubate 16-20 hours at room temperature						
Wash strips 5 times & discard liquid from the wells						
Ellman's reagent	200					
Incubate with an orbital shaker in the dark at RT						
Read the plate between 405 and 414 nm						

Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank wells (absorbance of Ellman's reagent alone) from the absorbance readings of the rest of the plate. If not, do it at this step.

- Subtract the average absorbance of NSB for each B0, standards, quality control and samples.

- Calculate the average absorbance for B0, standard, quality control and sample wells.

- Calculate B/B0 (%) for each standard, QC and sample (average absorbance of standards, QC or sample divided by average absorbance of B0) & multiply by 100.

- Using a semi-log graph paper for each standard point,

plot the B/B0 (%) on y axis versus the concentration on x axis. Draw a best-fit line through the points.

- To determine the concentration of your sample, the corresponding B/B0 (%) value has to be comprised between 20% and 80%. Find the B/B0 (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.

- Most plate readers are supplied with curve-fitting software capable of graphing these data (4-parameter or 5-parameter logistic fit). If you have this type of software, we recommend using it. Refer to it for further information.

Two vials of Quality Control are provided with this kit.
Your standard curve is validated only if the calculated concentration of the Quality Control obtained with the assay is +/- 25% of the expected concentration (written on the label of the QC vial)

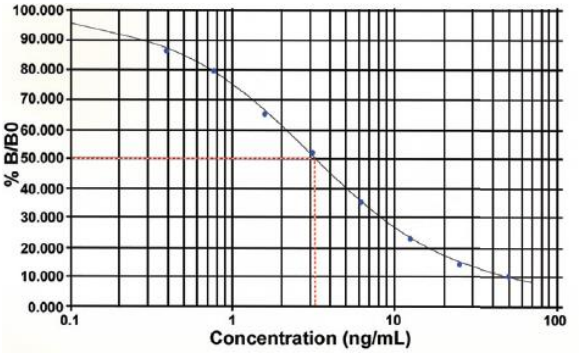
Acceptable range

- B0 absorbance > 0.200 A.U. blank subtracted in the conditions indicated above.
- NSB absorbance / B0 absorbance: < 0.1
- IC50: 2.0 to 3.4 ng/mL (mean: 2.8 ng/mL)
- QC sample: ± 25% 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.
The data were obtained using all reagents as supplied in this kit under the following conditions: 90 minutes developing at +20°C, reading at 414 nm. A 4-PL fitting was used to determine the concentrations.

Standard	Absorbance (A.U.)	Concentration obtained (ng/mL)	B/B0 (%)
S1	0.049	49.7	50.0
S2	0.073	24.8	25.0
S3	0.110	11.9	12.5
S4	0.155	6.2	6.3
S5	0.206	3.4	3.1
S6	0.269	1.6	1.6
S7	0.327	0.7	0.8
S8	0.354	0.4	0.4
QC	0.279	3.1	3.0 – 5.0
B0	0.407	-	-



Troubleshooting

> *Absorbance values are too low:*

- organic contamination of water
- one reagent has not been dispensed
- incorrect preparation/dilution
- assay performed before reagents reached RT
- 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

- ***IC50 or QC concentrations not within the expected range:*** wrong preparation of standards
- ***Analyses of two dilutions of a biological sample do not agree:*** Interfering substances are present. Sample must be purified prior to EIA analysis (excepting plasma samples).
- ***If a plate is accidentally dropped after dispatch of the AChE substrate (Ellman's Reagent) or if it needs to be revealed again:***

- one only needs to wash the plate, add fresh Ellman's reagent and proceed with a new development
- otherwise, the plate can be stored at +4°C with Wash Buffer in wells while waiting for technical advice from the Bioreagent Department.

These are a few examples of troubleshooting that may occur. If further information or explanation is needed, please contact Bertin Bioreagent Technical Support by phone on +33 (0)139 306 036, fax +33 (0)139 306 299 or by E-mail tech@bertin-bioreagent.com. Please have batch number of the kit (see outside the box) ready to provide to the technical support.

Bertin Bioreagent offers ELISA Training kit #B05005. Feel free to contact our Technical Support. We are always happy to hearing from you.

Bibliography

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6. Valentin MA, Ma S, Zhao A, Legay F & Avrameas A. Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies. *J Pharm Biomed Anal.* **55**, 869–877 (2011).
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9. Miyoshi, T. *et al.* Involvement of bone morphogenetic protein-4 in GH regulation by octreotide and bromocriptine in rat pituitary GH3 cells. *Journal of Endocrinology* **197**, 159–169 (2008).

Additional readings

List of publications quoting the use of this kit

Pujianto D.A, Curry B.J. and Aitken R.J.
Prolactin Exerts a Prosurvival Effect on Human Spermatozoa via Mechanisms that Involve the Stimulation of Akt Phosphorylation and Suppression of Caspase Activation and Capacitation.
Endocrinology, March 2010, 151(3):1269–1279

Kanisra Mary F., Eswaramohan T., Surendran S.N. *et al*.
Mukkuddu Maathrai (Triple Tablet), An Indigenous Medicine Elevates Serum Prolactin Level Of Female Rats (*Rattus norvegicus*).
IOSR Journal Of Pharmacy, Volume 3, Issue (January 2013), PP.38-42

Capasso R.
Effect of Silitidil, a Standardized Extract of Milk Thistle, on the Serum Prolactin Levels in Female Rats.
Natural Product Communications Vol. 9 (7) 2014

Suzuki Y., Nakahara K., Maruyama K. *et al*.
Changes in mRNA expression of arcuate nucleus appetiteregulating peptides during lactation in rats.
Journal of Molecular Endocrinology; April 1, 2014; vol.52; 97-109

Nikishina Y.O., Sapronova A.Y., Ugrumov M.V.
The Effect of Dopamine Secreted by the Brain into the Systemic Circulation on Prolactin Synthesis by the Pituitary gland in Ontogenesis.
Acta Naturae, vol. 8 № 3 (30) 2016

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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.

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

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