



PROLACTIN (rat)

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European patent # 89 139 552

U.S. patent # 50 47 330

**Prolactin (rat)
Enzyme Immunoassay kit
#A05101.96 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

#A11101
Version: 0217

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

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Mouse anti-rabbit IgG 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
EIA 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	-	A11101.1 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 33 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.

▶ **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

The QC samples provided in this kit have been prepared by diluting rat plasma (Sprague Dawley rat) in EIA 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 contains less than 100 µg of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

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

▷ Acetylcholinesterase AChE® Technology

Acetylcholinesterase (AChE®), the enzymatic label for EIA, 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 is capable of massive catalytic turnover during the generation of the electrochemical discharges. The use of AChE as enzymatic label for EIA has been patented by the French academic research Institute CEA [1, 2, 3], and Bertin Pharma, formerly known as SPI-Bio, has expertise to develop and produce EIA 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 and can be read at 405-414 nm. AChE® offers several advantages compared to enzymes conventionally used in EIAs:

- **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 Horseradish Peroxidase (HRP) or alkaline phosphatase. AChE® allows a greater sensitivity than other labeling enzymes.
- **Low background:** non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent. So, AChE® allows a very low background and an increased signal/noise ratio compared to other substrate of enzymes which is inherently unstable.

- > **Wide dynamic range:** AChE[®] is a stable enzyme and its activity remains constant for many hours as, 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) 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.

▷ 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

This Enzyme Immunometric Assay (EIA) 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.

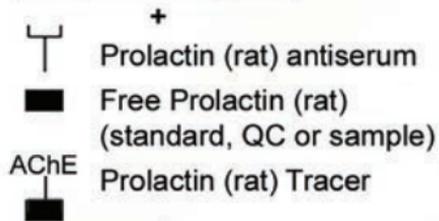
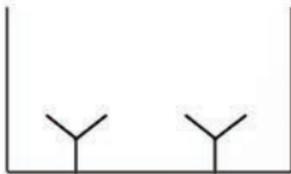
The complex rabbit antiserum-rat Prolactin (free Prolactin or Tracer) binds to the mouse monoclonal anti-rabbit antibody coating the well.

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

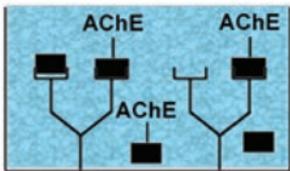
AChE acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm. The intensity of the colour, determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat Prolactin present in the well during the immunological incubation.

The principle of the assay is summarised below:

Wells coated with mouse IgG against rabbit

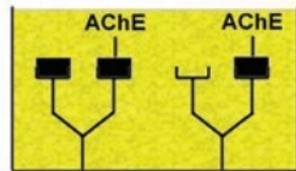


Immunological reaction



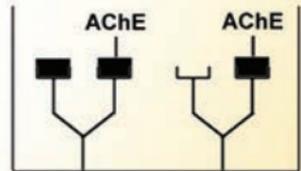
Incubation →

Revelation



↑
Ellman's Reagent

Washing



► **Materials and equipment required**

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

- Precision micropipettes (20 to 1000 μL)
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300 μL
- UltraPure water #A07001.1L
- Polypropylene tubes



Water used to prepare all EIA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).

Otherwise, 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 Pharma: 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, EDTA, 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 [5, 6].

▶ 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 33 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 to the assay.

▶ EIA Buffer

Reconstitute the vial #A07000 with 50 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 month.

▶ Prolactin (rat) Standard

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

The concentration of the first standard (S1) is 50 ng/mL.

Prepare seven polypropylene tubes for the other standards (S2 to S8) and add 500 µL of EIA buffer into each tube. Then prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of Assay Buffer	Standard concentration pg/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 EIA 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 EIA 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 at +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 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 and place the unused strips back in the packet.

Stability at +4°C: 1 month.

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.

	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

B0: Maximum Binding

NSB : Non Specific Binding

S1-S8 : Standards 1-8

* : QC : Samples or Quality Controls

▶ 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, 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.

> **EIA Buffer**

Dispense 100 μL to Non Specific Binding (NSB) wells and 50 μL to Maximum Binding (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 EIA 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 +4°C.

▷ **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 5 times with 300 μL of 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 200 μL of Ellman's Reagent to each well. Cover the plate with an aluminum sheet and incubate in the dark at room temperature, on an orbital shaker.
- > 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 0.2 A.U. (blank subtracted).**

Enzyme Immunoassay Protocol (volumes are in μL)						
Volume	Wells	Blank	NSB	B0	Standard	Sample or QC
EIA Buffer		-	100	50	-	
Standard		-	-	-	50	-
Sample or QC		-	-	-	-	50
Tracer		-	50	50	50	50
Antiserum		-	-	50	50	50
Cover plate, incubate 16-20 hours at $+4^{\circ}\text{C}$						
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 it is not the case, please do it.

- Subtract the average absorbance of NSB for each B₀, standards, QC and samples.
- Calculate the average absorbance for each B₀, standard, QC and sample.
- Calculate the B/B₀ (%) for each standard, QC and sample (average absorbance of standard, QC or sample divided by average of B₀) & multiplied by 100.
- For each standard, using a semi-log graph paper, 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 samples, the corresponding B/B₀ (%) value has to fall within the linear range of the standard curve (usual range of 20%-80%). Find the B/B₀ (%) value of each sample 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 a curve-fitting software capable of graphing these data (4-parameter logistic fit 4PL). 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 (see the label of QC vial)

▶ **Acceptable range**

- > B0 absorbance >0.200 A.U. blank subtracted in the conditions indicated above.
- > Ratio NSB absorbance / B0 absorbance: <0.1 mAU
- > IC50: 2.0 to 3.4 ng/mL (mean: 2.8 ng/mL)
- > QC sample: $\pm 25\%$ of the expected concentration (see the label on QC vial)

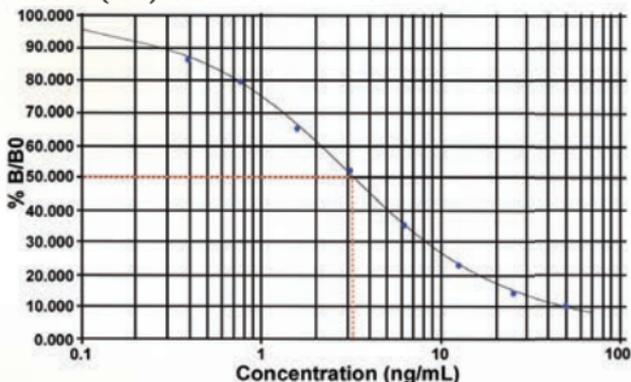
► Typical results

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: 60 minutes developing at +20°C, reading at 414 nm. A four-parameter logistic fitting was used to determine the concentrations.

Prolactin (rat)	Absorbance (A.U.)	Concentration obtained (ng/mL)	Concentration expected (ng/mL)
Standard 1	0.049	49.7	50.0
Standard 2	0.073	24.8	25.0
Standard 3	0.110	11.9	12.5
Standard 4	0.155	6.2	6.3
Standard 5	0.206	3.4	3.1
Standard 6	0.269	1.6	1.6
Standard 7	0.327	0.7	0.8
Standard 8	0.354	0.4	0.4
QC	0.279	3.1	3.0 - 5.0
BO	0.407	-	-

Typical Prolactin (rat) standard curve



► Assay validation and characteristics

The Enzyme Immunometric assay of Prolactin (rat) has been validated by Duhau *et al.* for its use in rat plasma [7].

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography [8, 9].

- **Limit of detection** (LOD) calculated as the concentration of Prolactin corresponding to the BO average minus three standard deviations: 0.2 ng/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 assay (ie. 30 assays per QC):

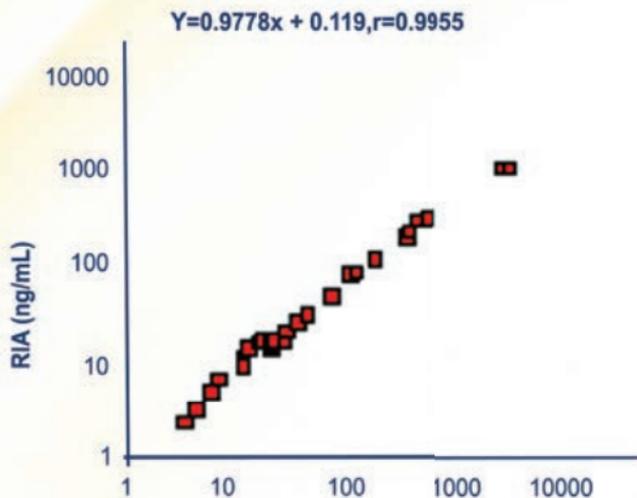
	Plasma QC (1 ng/mL)	Plasma QC (4.6 ng/mL)	Plasma QC (11.5 ng/mL)
Mean value	1.15	4.60	11.7
Number of values	30	30	30.0
Intra-assay coefficient of variation (%)	11.5	10.6	10.0
Inter-assay coefficient of variation (%)	13.4	14.8	19.3
Recovery (%) ± confidence intervalle	112.5 ± 5.4	89.5 ± 9.5	102.8 ± 7.5

- **Cross-reactivity** with rat LH, rat GH & rat TSH: <1%.

> Accuracy

Prolactin added	Prolactin measured	Recovery	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)

► Troubleshooting

- > **Absorbance values are too low:**
 - organic contamination of water,
 - one reagent has not been dispensed,
 - incorrect preparation / dilution,
 - assay performed before reagents reached room temperature,
 - 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 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

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Aluminium-induced changes of fusion pore properties attenuate prolactin secretion in rat pituitary lactotrophs.
Neuroscience 201 (2012), 57-66
6. Miyoshi T., Otsuka F., Otani H. *et al.*
Involvement of bone morphogenetic protein-4 in GH regulation by octreotide and bromocriptine in rat pituitary GH3 cells.
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J Pharm Biomed Anal. (2011) 55(5) : 869-877
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Guideline on bioanalytical method validation, 21 July 2011

Additional readings

List of publications quoting the use of this kit

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Acta Naturae, vol. 8 Nº 3 (30) 2016
11. Suzuki Y., Nakahara K., Maruyama K. *et al.*
Changes in mRNA expression of arcuate nucleus appetite-regulating peptides during lactation in rats.
Journal of Molecular Endocrinology; April 1, 2014; vol.52; 97-109

12. Capasso R.
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Natural Product Communications Vol. 9 (7) 2014

13. 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

14. 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

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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.

We are able to provide you with local technical support to use at ease our products.

For further information, please send your request to
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