

**BioVendor**

Research  
and Diagnostic Products



## **RAT PROLACTIN ELISA**

**Product Data Sheet**

**Cat. No.: RTC006R**

**For Research Use Only**

## CONTENTS

---

1.	INTENDED USE	3
2.	STORAGE, EXPIRATION	3
3.	INTRODUCTION	3
4.	TEST PRINCIPLE	4
5.	PRECAUTIONS	5
6.	TECHNICAL HINTS	6
7.	REAGENT SUPPLIED	6
8.	MATERIAL REQUIRED BUT NOT SUPPLIED	7
9.	PREPARATION OF REAGENTS	7
10.	SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS	7
11.	ASSAY PROCEDURE	8
12.	CALCULATIONS	9
13.	LIMITATIONS	10
14.	PERFORMANCE CHARACTERISTICS	10
15.	EXPECTED NORMAL VALUES	12
16.	REFERENCES	13
17.	SHORT INSTRUCTION	14

**»» This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

**»» Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

---

The BioVendor Rat Prolactin ELISA is an enzyme-immunoassay for the quantitative measurement of prolactin in rat serum.

## 2. STORAGE, EXPIRATION

---

When stored at 2°C to 8°C all reagents are stable until expiration date or 30 days after opening.

The Stop Solution is stable up to 2 months after opening or until the expiration date.

The Wash Buffer is stable for 3 months after dilution or until the expiration date.

Store Calibrators refrigerated, they will be stable at 2°C to 8°C for 7 days after reconstitution or until expiration date. For longer storage freeze at -20°C.

Protect Divisible Microplate from moisture. Store together with desiccant and carefully sealed in the plastic bag.

Protect TMB-Substrate Solution from light.

## 3. INTRODUCTION

---

Rat prolactin (rPRL) is a single-chain polypeptide hormone of the rat anterior pituitary with a molecule mass of approximately 23,000. Prolactin from different species exhibits significant variations in the amino acid sequence. Rat prolactin differs from human prolactin at about 50 percent of all residues.

The secretion of rPRL from the pituitary is inhibited by hypothalamic prolactin-inhibitory factor (PIF). Although dopamine was long thought to be this PIF molecule, today it seems that there is a special peptide with prolactin-inhibiting activities.

The release of prolactin is certainly stimulated by different peptides, particularly thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP). There is also evidence that rat posterior pituitary lobe contains a special prolactin releasing hormone.

The most important role of prolactin is stimulation of mammary gland growth and lactation. During pregnancy, blood prolactin levels climb, but the increases can differ enormously between rats. High prolactin levels are observed during lactation. Prolactin has a wide variety of other physiological actions, for example on the ovary. In the rat, prolactin has a luteotrophic effect which is not seen in many other species. Furthermore, prolactin is a stress hormone.

In rats, as in humans, prolactin exhibits a sleep-related diurnal variation. Peak values are seen in the late afternoon and nadir values in the morning.

Because of the variety of its actions, prolactin is one of the preferred hormones to monitor when testing the influence of new therapeutic agents and drugs on the endocrine system in the rat.

#### 4. TEST PRINCIPLE

---

The BioVendor Rat Prolactin ELISA kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of rat prolactin. The microplate is coated with a first monoclonal antibody specific for rat prolactin.

Calibrators and samples are pipetted into the antibody coated microplate. During a 2 hours incubation endogenous rat prolactin in the sample bind to the antibodies fixed on the inner surface of the wells. Non-reactive sample components are removed by a washing step.

Afterwards, a second polyclonal horseradish peroxidase-labeled antibody, directed against another epitope of the prolactin molecule, is added. During another 1 hour incubation, a sandwich complex consisting of the two antibodies and the rat prolactin is formed. An excess of enzyme conjugate is washed out.

A chromogenic substrate, TMB (3,3',5,5'-Tetra-Methyl-Benzidine), is added to all wells. During a 30 minutes incubation, the substrate is converted to a colored end product (blue) by the fixed enzyme. Enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution (change from blue to yellow). The color intensity is direct proportional to the concentration of rat prolactin present in the sample.

The optical density of the color solution is measured with a microplate reader at 450 nm. Bichromatic measurement with a 600 - 690 nm reference filter is recommended.

## 5. PRECAUTIONS

---

All reagents of this test kit are strictly intended for veterinary research use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.

Please adhere strictly to the sequence of pipetting steps provided in this protocol.

All reagents should be stored refrigerated at 2-8°C in their original container. Do not interchange kit components from different lots and assays. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components beyond their expiration dates.

Allow all kit components and specimen to reach room temperature (18–28°C) prior to use and mix well.

During handling of all kit reagents, control and serum samples observe the existing legal regulations handling potentially infectious materials. Especially the following precautions should be taken:

- do not eat, drink or smoke
- do not pipette by mouth, use safety pipettes
- wear disposable gloves and avoid contact with kit reagents, control and sample material.

The test kit contains components of veterinary origin which were found negative for Hepatitis B surface antigen and HIV (Human Immunodeficiency Virus). Nevertheless, for products derived from human or animal source it cannot be completely guaranteed, that they do not contain the above mentioned, others and not yet known or not diagnosticable pathogens. Sample material of patients (for example serum or plasma) normally used in laboratory determinations are always classified as potentially infectious. According to the same safety guides, kit reagents and control material are to be used. Samples of risk patients should be specially labeled and if necessary be handled in safety work benches (lamina flow bench).

The assay reagents contain against microbial growth preservation substances, avoid contact with skin and/or mucous membranes.

Avoid contact with the TMB (3,3',5,5'-Tetra-Methyl-Benzidine) substrate solution containing peroxide. If it comes into contact with skin, wash thoroughly with water. Avoid contact with any easily oxidized materials. Extreme temperature changes may cause spontaneous decay of the peroxide. Avoid the contact with the stop solution containing acid. By skin contact, wash thoroughly with water. All instrumentation employed to dispense the stop solution should be thoroughly cleaned after use.

## 6. TECHNICAL HINTS

---

- Do not interchange components of different lots.
- All components should be at room temperature (18–28°C) before use.
- All components of this test kit, supplied as concentrate should be diluted to their final concentration at least 30 minutes prior to use. Mix well, but prevent of foam formation. Use a disposable-tip micropipette to dispense serum samples. Pipet directly to the bottom of the wells. Change the tip between samples, to avoid carryover contamination.
- For internal quality control we suggest to use
- **Rat Control Set coded RTC900R. For more information please contact BioVendor.**

## 7. REAGENT SUPPLIED

---

1. **Divisible microplate**, 12 x 8 (break apart) strips with 96 wells, ready to use  
Removable wells coated with a monoclonal anti-rat prolactin antibody.
2. **Rat Prolactin Master Calibrator**, 1 vial, 80 ng, lyophilized,  
in serum/buffer matrix containing highly purified rat prolactin,  
**For reconstitution see “Reagent preparation”.**
3. **Rat Prolactin Calibrator/Sample Diluent**, 1 vial, 6 ml, ready to use  
rat prolactin free
4. **Enzyme-Labeled Rat Prolactin Antibody**, 1 vial, 22 ml, red, ready to use  
containing horseradish peroxidase-labeled polyclonal anti rat prolactin antibody  
in a buffered solution with preservative
5. **Rat Prolactin Sample Buffer**, 1 vial, 6 ml, yellow, ready to use
6. **TMB-Substrate Solution**, 1 vial, 22 ml, ready to use  
3,3',5,5'-Tetra-Methyl-Benzidine in buffered peroxide solution
7. **Stop Solution**, 1 vial, 7 ml, ready to use  
containing 2 M hydrochloric acid
8. **Wash Buffer Concentrate**, 1 vial, 50 ml, 10 x concentrated,  
**See “Reagent preparation”.**

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

---

- Microplate reader capable for endpoint measurements at 450 nm (optional reference filter in the range of 600 - 690 nm)
- Vortex mixer
- Microplate mixer operating at 900 rpm
- Distilled or deionized water
- Graduated cylinder for 500 ml
- Plastic containers for storage of the wash solution
- Adjustable pipette for up to 1000  $\mu$ l
- Dispenser or repeatable pipet for 25  $\mu$ l, 50  $\mu$ l, and 200  $\mu$ l

## 9. PREPARATION OF REAGENTS

---

### **Calibrators:**

Reconstitute lyophilized Rat Prolactin Master Calibrator with **1 ml dest. water** 30 min. before use (end concentration of 80 ng/ml). Make a dilution series with Calibrator/Sample Diluent to get calibrators with 80, 40, 20, 10 and 5 ng/ml.

### **Wash Buffer:**

Dilute with 450 ml dist. water to a final volume of 500 ml.

## 10. SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

---

For determination of rat prolactin serum is the preferred sample matrix. The procedure calls for 25  $\mu$ l matrix per well.

Prolactin is one of the most sensitive stress hormones of the rat. Blood collection should therefore be as stress-free as possible.

The samples may be stored refrigerated at 2–8°C for one week, or up to 2 months frozen at –20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Samples expected to contain rat prolactin concentrations higher than the highest calibrator (80 ng/ml) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results.

## 11. ASSAY PROCEDURE

### 1. Preparation of calibrators:

Label four tubes: E (40 ng/ml), D (20 ng/ml), C (10 ng/ml) and B (5 ng/ml). Pipet **0.1 ml** of the Calibrator/Sample Diluent into all tubes. Pipet 0.1 ml of the reconstituted Rat Prolactin Master Calibrator into tube E (40 ng/ml), and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Rat Prolactin Calibrator will serve as the highest calibrator F (80 ng/ml). Use the Rat Prolactin Calibrator/Sample Diluent as the zero Calibrator A (0 ng/ml).

	1	2	3	4	5	6	7	8	9	10	11	12
a	A	E	P3	P..								
b	A	E	P3	P..								
c	B	F	P4									
d	B	F	P4									
e	C	P1	P5									
f	C	P1	P5									
g	D	P2	P6									
h	D	P2	P6									

- Pipet **25 µl** of each calibrator, control and patient sample into the wells prepared.
- Add **50 µl** of **Rat Prolactin Sample Buffer** to every well.
- Shake for **2 hours** at room temperature (18-28°C). The mixer should be set at > 900 rpm.
- Discard the content of the wells and wash **4 times** with **300 µl** buffered wash solution. Remove as much wash solution as possible by beating the microplate carefully.
- Add **200 µl** of **Enzyme-Labeled Anti-Rat Prolactin Antibody** to all wells.
- Shake again for **1 hour**.
- Discard the content of the wells and wash **4 times** with **300 µl** buffered wash solution. Remove as much wash solution as possible by beating the microplate carefully.
- Add **200 µl** of liquid **TMB/Substrate Solution** to all wells.
- Incubate without shaking for **30 minutes** in the dark.
- Add **50 µl** of **Stop Solution** to each well and mix carefully.
- Read the optical density at **450 nm**. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

**The developed color is stable for at least 30 minutes. Read optical densities during this time.**



## 12. CALCULATIONS

For evaluation of rat prolactin a 4-Parameter-Fit with lin-log coordinates for optical density (linear scale) and concentration (logarithmic scale) is recommended.  
Spline approximation with lin-log coordinates and log-log coordinates are also suitable.

### Example of typical calibrator curve

The figure below shows typical results for BioVendor Rat Prolactin ELISA test kits. These data are intended for illustration only and should not be used to calculate results from another run.

	Replicate (OD)	Mean (OD)	Binding (%)	Rat Prolactin (ng/mL)
<b>Calibrators</b>				
<b>A</b>	0.079 ----- 0.067	0.073	-	<b>0</b>
<b>B</b>	0.189 ----- 0.169	0.179	<b>6.9</b>	<b>5</b>
<b>C</b>	0.328 ----- 0.357	0.342	<b>12.7</b>	<b>10</b>
<b>D</b>	0.766 ----- 0.827	0.796	<b>29.7</b>	<b>20</b>
<b>E</b>	1.591 ----- 1.679	1.635	<b>61.0</b>	<b>40</b>
<b>F</b>	2.588 ----- 2.765	2.677	<b>100</b>	<b>80</b>
<b>Unknown Samples</b>				
<b>X 001</b>	0.318 ----- 0.329	0.324	12,1	9.8
<b>X 002</b>	0.577 ----- 0.603	0.590	22,0	16.4
<b>X 003</b>	1,733 ----- 1,698	1,716	64,1	47.3

## 13. LIMITATIONS

---

### 13.1 Effect of Anticoagulants

To determine whether anticoagulants interfere with the assay, blood was collected from 30 rats into plain and EDTA vacutainer tubes. All samples were assayed by the BioVendor Rat Prolactin ELISA procedure, with the following results.

(EDTA) = 1.05 (Serum) – 9.3 ng/ml r = 0.978

Means: 3.68 ng/ml (Serum)  
3.78 ng/ml (EDTA)

A limited study with citrated and heparinized plasma show comparable results to EDTA plasma.

### 13.2 "High-Dose Hook"-Effect

Rat sera containing up to 300 ng/ml Prolactin were measured with the BioVendor Rat Prolactin ELISA assay. A High-Dose Hook effect could not be observed.

## 14. PERFORMANCE CHARACTERISTICS

---

### 14.1 Analytical sensitivity

The lower detection limit for rat prolactin is approximately 0.6 ng/ml.

### 14.2 Specificity

The antibodies in the BioVendor Rat Prolactin ELISA procedure are highly specific for rat prolactin. Detectable crossreactivities to other hormones that may be present in serum samples are not known.

The following substances were tested:

	added quantity (ng/ml)	measured concentration (ng/ml)	cross-reactivity (%)
Rat TSH	5,000	1.5	0.03
Rat FSH	10,000	5.1	0.5
Rat LH	5,000	nd	-
Rat GH	4,000	nd	-

### 14.3 Reproducibility

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 20 pairs of wells in a single run for Intra-Assay precision and the Inter-Assay precision was calculated from the results of 14 different runs of three samples:

Intra - Assay			
Serum No	Mean $\bar{x}$ (ng/ml)	SD $\pm s$ (ng/ml)	CK (%)
1	6.6	0.20	3.0
2	19.6	0.77	3.9
3	33.0	1.80	5.5

Inter - Assay			
Serum No	Mean $\bar{x}$ (ng/ml)	SD $\pm s$ (ng/ml)	CK (%)
1	6.5	0.28	4.3
2	20.5	0.71	3.5
3	33.4	1.48	4.4

### 14.4 Recovery

Three spiking solutions were prepared using the Sample Diluent, to represent the 600, 800 and 1,000 ng/ml, respectively. A 50  $\mu$ l aliquot of each solution (A, B, C) was spiked into 950  $\mu$ l aliquots of two different rat serum samples, for a spiking ratio of 1 to 20, leaving the serum matrix of the spiked samples relatively intact. All samples were then assayed by the BioVendor Rat Prolactin ELISA procedure.

#### BioVendor Rat Prolactin ELISA

Sample	Diluted Solution	measured Concentration [ng/ml]	expected Concentration [ng/ml]	Recovery [%]
1	-	26.1	-	-
	A	54.7	56.1	97
	B	66.6	66.1	100
	C	79.9	76.1	105
2	-	24.2	-	-
	A	52.6	54.2	103
	B	67.5	64.2	95
	C	81.1	74.2	91

## 14.5 Linearity

In dilution experiments sera with high rat prolactin concentrations were diluted with sample diluent and assayed in the BioVendor Rat Prolactin ELISA kit. The assay showed linearity over the full measuring range.

BioVendor Rat Prolactin ELISA

Sample	Dilution Factor	measured Concentration	expected Concentration	Recovery
		[ng/ml]	[ng/ml]	[%]
1	8 in 8	20.9	-	-
	4 in 8	10.6	10.5	101
	2 in 8	5.9	5.2	113
	1 in 8	3.0	2.6	115
2	8 in 8	34.7	-	-
	4 in 8	16.7	17.4	96
	2 in 8	9.0	8.7	103
	1 in 8	4.5	4.3	105

## 15. EXPECTED NORMAL VALUES

In a reference range study rat serum samples were collected in the morning between 8 and 9 a.m. and in the evening between 5 and 6 p.m. Diurnal variations have not been observed. Analysis by the BioVendor Rat Prolactin ELISA kit yielded the following results:

Group	Absolute Range (ng/ml)	n
Normal female rats	nd - 17.9	15
Normal male rats	nd- - 23.4	10
nd = nondetectable		

Because of differences which may exist between laboratories with respect of population, laboratory technique and selection of reference groups, it is recommended that each laboratory establishes its own normal and pathological ranges of rat prolactin. The reference ranges should be regarded as guidelines only.

## 16. REFERENCES

---

### »» References to prolactin:

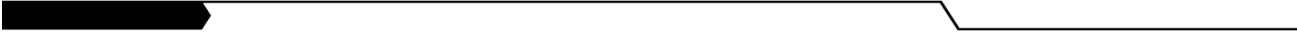
1. Leung, F. C., Russel, S. M.; Nicoll, C. S. Relationship between bioassay and radioimmunoassay estimates of prolactin in rat serum. *Endocrinology* 1978; 103: 1619 - 28.
2. Martinat, N., Hall, E., Ravault, J. P., Dubois, M. P. Purification of rat prolactin: development of an homologous radioimmunological assay and comparison with the NIAMDD system. *Ann Biol Anim Bioch Biophys* 1979; 19: 1771 - 48.
3. Beach, J. E., Miles, D. J., Lukes, Y. G., Vigersky, R. A. Microplate solid-phase radioimmunoassay for rat prolactin. *J Lab Clin Med* 1985; 105: 294 - 298.
4. Butcher, R. L., Collins, W. E., Fugo, N. W. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17 $\beta$  throughout the 4-day estrous cycle of the rat. *Endocrinology* 1974; 94: 1704 - 8.
5. Barbieri, R. L., Todd, R. B., Morishita, H., Ryan, K. J., Fishman, J, Naftolin, F. Response of serum prolactin to catechol estrogen in the immature rat. *Fertil Steril* 1980; 34: 391 - 3.
6. Wong, C. C. Endogene und exogene Einflüsse auf die Variabilität der Hormonausschüttung bei der Ratte (Endogenous and exogenous influences on the variability of hormone release in the rat). Thesis, Hannover (Germany): Univ. of Hannover, 1981.
7. Campbell, G. A., Kurcz, M., Marshall, S., Meites, J. Effects of starvation in rats on serum levels of FSH, LH, TSH, growth hormone and prolactin: response to LH-releasing hormone and thyrotropin-releasing hormone. *Endocrinology* 1977; 100: 580 - 7.
8. Haggi, E., Aoki, A. Prolactin content in rat pituitary gland. RIA of prolactin after different extraction procedures. *Acta Endocrinol* 1981; 97: 338 - 42.
9. Reichel, J. Wirkungen gonadaler Steroide auf die adeno-hypophysären Thyreotropin-Releasing-Hormon-Rezeptoren, den Prolactinserumspiegel und die hormonelle Hypophysen-Schilddrüsen-Achse der Ratte (Effects of gonadal steroids on the pituitary TRH-receptors, the serum prolactin concentrations and the pituitary-thyroid axis in the rat) Thesis. Lübeck (Germany): University of Lübeck, 1990.
10. Moishige, W. K., Pepe, G. J., Rothschild, I. Serum LH, prolactin and progesterone levels during pregnancy in the rat. *Endocrinology* 1973; 92: 1527 - 30.

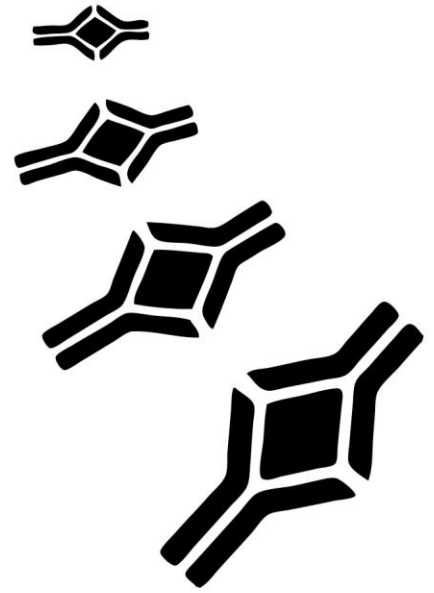
## 17. SHORT INSTRUCTION

(all sample sizes given in  $\mu\text{l}$ )

MP Well	ng/ml	0	1	2	3	4	5	Sample
		0	5	10	20	40	80	
Steps	Solution							
Pipet	Calibrator	25	25	25	25	25	25	-
Pipet	Sample	-	-	-	-	-	-	25
Pipet	Rat Prolactin	50	50	50	50	50	50	50
	Sample Buffer							
Incubate for 2 hours at RT on a shaker								
Decant								
Wash 4x with 300 $\mu\text{l}$ of buffered wash solution								
Pipet	Enzyme-labeled Rat Prolactin Ab	200	200	200	200	200	200	200
Incubate for 1 hour at RT on a shaker								
Decant								
Wash 4x with 300 $\mu\text{l}$ of buffered wash solution								
Pipet	Substrate Solution	200	200	200	200	200	200	200
Incubate for 30 min at RT in the dark								
Pipet	Stop Solution	50	50	50	50	50	50	50
Read at $\lambda = 450 \text{ nm}$								

NOTES





**BioVendor – Laboratorní medicína, a.s.**

Karasek 1767/1, 621 00 Brno, Czech Republic

Phone: +420-549-124-185, Fax: +420-549-211-460

E-mail: [info@biovendor.com](mailto:info@biovendor.com), [sales@biovendor.com](mailto:sales@biovendor.com)

Web: [www.biovendor.com](http://www.biovendor.com)

There are BioVendor branches and distributors near you.

To find the office closest to you, visit [www.biovendor.com/contact](http://www.biovendor.com/contact)

