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Instructions for use

Prolactin rat ELISA

REF AR E-8200R

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Prolactin rat ELISA

1. INTRODUCTION

1.1. Intended Use

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

1.2 Summary and Explanation

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.

2. PRINCIPLE

The *Prolactin rat 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.
Standards 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. Bi-chromatic measurement with a 600 - 690 nm reference filter is recommended.

3. WARNINGS AND PRECAUTIONS

1. This kit is strictly intended for *research use only*. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.

9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided

AR E- 8231 TUT 96 **Microtiterplate** - Ready to use

Contents: 12 x 8 (break apart) strips with 96 wells.
Removable wells coated with a monoclonal anti-rat prolactin antibody.

AR E-8201 STANDARD **Rat Prolactin Master Standard** - Lyophilized

Contents: in serum/buffer matrix containing highly purified rat prolactin.

Volume: 1 x 80 ng

For reconstitution see "Reagent Preparation".

AR E-8260 DILUENT **Rat Prolactin Standard/Sample Diluent** - Ready to use

Contents: rat prolactin free.

Volume: 1 x 6 ml

AR E-8240 CONJUGATE **Enzyme-Conjugate** - Ready to use

Contents: containing horseradish peroxidase-labeled polyclonal anti rat prolactin antibody in a buffered solution with preservative. Red.

Volume: 1 x 22 ml

AR E-8270 SAMPLE-BUFF **Rat Prolactin Sample Buffer** - Ready to use

Contents: yellow

Volume: 1 x 6 ml

AR E-0055 SUBSTRATE **Substrate Solution** - Ready to use

Contents: 3,3',5,5'-Tetra-Methyl-Benzidine (TMB) in buffered peroxide solution.

Volume: 1 x 22 ml

Hazards identification:

H360D May damage the unborn child.

AR E-0030 WASH-CONC 10x **Wash Solution** - 10x concentrated

Volume: 1 x 50 ml

See "Reagent Preparation".

AR E-0080 STOP-SOLN **Stop Solution** - Ready to use

Contents: contains 2 M Hydrochloric Acid

Volume: 1 x 7 ml

Hazards identification: 

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

4.2 Materials required but not provided

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

4.3 Reagent preparation

Standards:
Reconstitute lyophilized Master Standard with 1 ml dest. water 30 min. before use (end concentration of 80 ng/ml). Make a dilution series with Standard/Sample Diluent to get standards with 80, 40, 20, 10 and 5 ng/ml.

Wash Buffer:
Dilute with 450 ml dist. water to a final volume of 500 ml.
The diluted Wash Solution is stable for 12 weeks at room temperature.

4.4 Storage conditions

When stored at 2°C to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2° - 8 °C. After first opening the reagents are stable for 30 days if used and stored properly.
Microtiter wells must be stored at 2°C to 8 °C. Take care that the foil bag is sealed tightly. Store Standards refrigerated, they will be stable at 2°C to 8 °C for 7 days after reconstitution. For longer storage aliquot and freeze at -20 °C. Protect Substrate Solution from light.

5. SPECIMEN

For determination of rat prolactin serum is the preferred sample matrix. The procedure calls for 25 µl 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 Standard (80 ng/ml) should be diluted with Standard/Sample Diluent before assay. The additional dilution step has to be taken into account for the calculation of the results.

6. ASSAY PROCEDURE

6.1 General remarks

- 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 AR K-8000. For more information please contact the manufacturer.**

6.2 Assay Procedure

1 Preparation of Standards

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 Standard/Sample Diluent into all tubes. Pipet 0.1 ml of the reconstituted Master Standard into tube E (40 ng/ml), and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Standard will serve as the highest Standard F (80 ng/ml). Use the Standard/Sample Diluent as the zero Standard 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									

2.	Pipet **25 µl** of each **standard, control** and **sample** into the wells prepared.
3.	Add **50 µl** of **Sample Buffer** to every well.
4.	**Rotate** for **2 hours at room temperature** (18 - 28 °C) on a plate mixer (600 - 900 rpm).
5.	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.
6.	Add **200 µl** of **Enzyme Conjugate** to all wells.
7.	Shake again for **1 hour** (600 – 900 rpm).
8.	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.
9.	Add **200 µl** of liquid **Substrate Solution** to all wells.
10.	Incubate without shaking for **30 minutes in the dark**.
11.	Add **50 µl** of **Stop Solution** to each well and mix carefully.
12.	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.

6.2 Calculation of Results

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.

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6.3.1 Example of typical Standard Curve

The figure below shows typical results for *Prolactin rat 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)
Standards				
A	0.079 0.067	0.073	-	0
B	0.189 0.169	0.179	6.9	5
C	0.328 0.357	0.342	12.7	10
D	0.766 0.827	0.796	29.7	20
E	1.591 1.679	1.635	61.0	40
F	2.588 2.765	2.677	100	80
Unknown Samples				
X 001	0.318 0.329	0.324	12.1	9.8
X 002	0.577 0.603	0.590	22.0	16.4
X 003	1.733 1.698	1.716	64.1	47.3

7. 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 *Prolactin rat 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.

8. PERFORMANCE CHARACTERISTICS

8.1 Analytical Sensitivity

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

8.2 Specificity

The antibodies in the *Prolactin rat 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	-

8.3 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			
Sample	Mean $\bar{x}$ (ng/ml)	SD ± 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			
Sample	Mean $\bar{x}$ (ng/ml)	SD ± 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

8.4 Recovery

Three spiking solutions were prepared using the Sample Diluent, to represent the 600, 800 and 1,000 ng/ml, respectively. A 50 µl aliquot of each solution (A, B, C) was spiked into 950 µl 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 *Prolactin rat ELISA* procedure.

Sample	**Diluted Solution**	**Measured Concentration**	**Expected concentration**	**Recovery**
		(ng/ml)	(ng/ml)	(%)
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

8.5 Linearity

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

Sample	**Diluted 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

9. LIMITATIONS OF PROCEDURE

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 *Prolactin rat 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 heparinzed plasma show comparable results to EDTA plasma.

"High-Dose Hook"-Effect

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

10. REFERENCES

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-17ß 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.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!