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Instructions for use

Prolactin rat ELISA

REF **AR E-8200**

96

+2 +8 °C

RUO For Research use only-
Not for use in diagnostic procedures

RAT PROLACTIN ELISA

1. INTENDED USE

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

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.

3. PRINCIPLE

The LDN 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.

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. Bi-chromatic measurement with a 600 - 690 nm reference filter is recommended.

4. WARNINGS AND 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 ℃ 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 ℃) 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.

5. REAGENTS

REAGENTS PROVIDED

AR E- 8231 TU 96 **Divisible microplate,** 12 x 8 (break apart) strips with 96 wells, ready to use
Removable wells coated with a monoclonal anti-rat prolactin antibody.

AR E-8201 STANDARD **Rat Prolactin Master Calibrator,** 1 vial, 80 ng, lyophilized,
in serum/buffer matrix containing highly purified rat prolactin,
For reconstitution see "Reagent preparation".

AR E-8260 DILUENT **Rat Prolactin Calibrator/Sample Diluent,** 1 vial, 6 ml, ready to use
rat prolactin free

AR E-8240 CONJUGATE **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

AR E-8270 SAMPLE-BUFF **Rat Prolactin Sample Buffer,** 1 vial, 6 ml, yellow, ready to use

AR E-0055 SUBSTRATE **Substrate Solution**, 1 vial, 22 ml each, ready to use;
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

AR E-0080 STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;
contains 2 N Hydrochloric Acid solution.

AR E-0030 WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10X concentrated);
see „Preparation of Reagents".

6. 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 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 µl
- Dispenser or repeatable pipet for 25 µl, 50 µl, and 200 µl

7. REAGENT PREPARATION

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.

8. STORAGE CONDITIONS

When stored at 2℃ to 8℃ all reagents are stable u ntil 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 at -20 ℃ or below (in aliquots), it will be stable for 7 days after reconstitution or until expiration date.
Protect Divisible Microplate from moisture. Store together with desiccant and carefully sealed in the plastic bag.
Protect TMB-Substrate Solution from light.

9. 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 ℃ for one week, or up to 2 months frozen at –20 ℃. T o 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.

10. ASSAY PROCEDURE

GENERAL REMARKS

- Do not interchange components of different lots.
- All components should be at room temperature (18 – 28 ℃) 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.

TEST 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									

2. Pipet **25 µl** of each calibrator and patient sample into the wells prepared.

3. Add **50 µl** of **Rat Prolactin Sample Buffer** to every well.

4. Shake for **2 hours** at room temperature (18 - 28 ℃). The mixer should be set at > 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-Labeled Anti-Rat Prolactin Antibody** to all wells.

7. Shake again for **1 hour**.

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 **TMB/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.

11. 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.

12. EXAMPLE OF TYPICAL CALIBRATOR CURVE

The figure below shows typical results for LDN 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)
Calibrators				
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

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 LDN 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.

13. PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY

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

SPECIFICITY

The antibodies in the LDN 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	-

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 ± 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 ± 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

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 LDN Prolactin rat ELISA procedure.

LDN Prolactin rat ELISA

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

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

LDN Prolactin rat 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

14. 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 LDN 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 LDN Prolactin rat ELISA assay. A High-Dose Hook effect could not be observed.

1. REFERENCES

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2. SHORT INSTRUCTION

(all sample sizes given in µl)

MP Well / Steps		0	1	2	3	4	5	Sample
	ng/ml	0	5	10	20	40	80	
	Solution							
Pipet	Calibrator	25	25	25	25	25	25	-
Pipet	Sample	-	-	-	-	-	-	25
Pipet	Rat Prolactin Sample Buffer	50	50	50	50	50	50	50
Incubate for 2 hours at RT on a shaker								
Decant Wash 4x with 300 µl 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 µl 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 λ = 450 nm								

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!