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Instructions for use

Prolactin canine ELISA

REF **AR E-8400**

2°C – 8°C

Σ 96

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

Prolactin canine ELISA

INTENDED USE

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

SUMMARY AND EXPLANATION

Canine prolactin (cPRL) is a single-chain polypeptide hormone of the canine anterior pituitary with a molecular mass of approx. 22,000. Prolactin from different species exhibits significant variations in the amino acid sequence. Canine prolactin differs from human prolactin at about 60 percent of all residues.
The secretion of cPRL 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 vasocative intestinal peptide (VIP). Estrogens and progesterone also seem to play a role in the secretion of prolactin, and neurogenic factors influence its release. Milking and suckling are immediately followed by an increase in serum cPRL.
The most important role of prolactin is stimulation of mammary gland growth and lactation. During pregnancy, prolactin levels in canine blood increase slightly; during lactation, significantly. Prolactin has a wide variety of other physiological actions. It affects water and electrolyte balance, metabolism and gonadal function; is an important stress hormone; and seems to play a role in the maintenance of the long interestrous interval in the bitch.
In dogs with pituitary-dependent hyperadrenocorticism, prolactin levels in blood were higher than in healthy animals. Prolactin determinations can be used in the therapeutic control of hyperprolactinemia. During a pseudo pregnancy, prolactin is increased. Therapy with alkaloids like bromocriptine lowers PRL levels, and lactation and maternal behaviour are decreased.
The secretory capacity of the pituitary can be tested with the TRH stimulation test.

PRINCIPLE

The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of canine prolactin. The microplate is coated with a first monoclonal antibody specific for canine prolactin.
Calibrators and samples are pipetted into the antibody coated microplate. During a 2 hours incubation endogenous canine 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 an 1 hour incubation, a sandwich complex consisting of the two antibodies and the canine 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 canine 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.

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 °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 animal 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 preservation substances against microbial growth, 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.

REAGENTS

Reagents provided

AR E-8431 96 **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells; ready to use
Wells coated with a monoclonal anti-canine prolactin antibody.

AR E-8401 STANDARD **Canine Prolactin Master Calibrator,** 1 vial, 80 ng, lyophilized;
in serum/buffer matrix containing highly purified canine prolactin;
Reconstitute lyophilized Canine Prolactin Master Calibrator with **1 ml dest. water** 30 min. before use (end concentration of 80 ng/ml). Make a dilution serie with Calibrator/Sample Diluent to get calibrators with 80, 40, 20, 10, 5 and 2.5 ng/ml.

AR E-8440 CONJUGATE **Enzyme-Labeled anti canine Prolactin Antibody,** 1 vial, 22 ml, red, ready to use; containing horseradish peroxidase labeled polyclonal anti canine prolactin antibody

AR E-8460 DILUENT **Canine Prolactin Calibrator/Sample Diluent,** 1 vial, 6 ml, ready to use;
canine prolactin free

AR E-8470 SAMPLE-BUFF **Canine 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;**
3,3',5,5'-Tetra-Methyl-Benzidine in buffered peroxide solution
AR E-0080 STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;
contains 2 M Hydrochloric Acid solution.

AR E-0030 WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10X concentrated);
Dilute with 450 ml dist. water to a final volume of 500 ml.

Note: Additional Calibrator 0 for sample dilution is available upon request.

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 cylinders 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.

STORAGE CONDITIONS

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 at -20 °C 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.

SPECIMEN

For determination of canine 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 dog. 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 canine prolactin concentrations higher than the highest calibrator (80 ng/ml) should be diluted with the Canine Prolactin Calibrator/Sample Diluent before assay. The additional dilution step has to be taken into account for the calculation of the results.

ASSAY PROCEDURE

GENERAL REMARKS

Do not interchange components of different lots.
All components should be at room temperature (18 – 28 °C) before use.
All components of these test kits, 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.

ASSAY PROCEDURE

1. Preparation of calibrators:
   Label five tubes: F (40 ng/ml), E (20 ng/ml), D (10 ng/ml), C (5 ng/ml), and B (2.5 ng/ml). Pipet **0.1 ml** of the Calibrator/Sample Diluent into all tubes. Pipet 0.1 ml of the reconstituted Canine Prolactin Master Calibrator into tube F (40 ng/ml) and mix thoroughly. Transfer 0.1 ml from tube F (40 ng/ml) to tube E (20 ng/ml) and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Canine Prolactin Calibrator will serve as the highest calibrator G (80 ng/ml). Use the Canine 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	P2	P..								
b	A	E	P2	P..								
c	B	F	P3									
d	B	F	P3									
e	C	G	P4									
f	C	G	P4									
g	D	P1	P5									
h	D	P1	P5									

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

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

4. Rotate for **2 hours** at room temperature (18 - 28 °C) on a plate mixer (350-400 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-canine 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 15 minutes. Read optical densities during this time.

CALCULATION OF RESULTS

For evaluation of canine 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 canine prolactin 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 (%)	canine prolactin (ng/ml)
Calibrators				
A	0.059 ---------- 0.055	0.057	-	0
B	0.148 ---------- 0.148	0.148	4.4	2.5
C	0.244 ---------- 0.299	0.272	8.1	5
D	0.495 ---------- 0.559	0.527	15.7	10
E	0.917 ---------- 0.953	0.935	27.8	20
F	1.996 ---------- 2.074	2.035	60.6	40
G (Bmax)	3.207 ---------- 3.507	3.357	100	80
Unknown Samples				
X 001	0.795 ---------- 0.772	0.784	23.3	15.8
X 002	1.703 ---------- 1.730	1.717	51.1	33.9
X 003	2.384 ---------- 2.446	2.415	71.9	50.0

EXPECTED NORMAL VALUES

In a reference range study canine 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 canine ELISA kit yielded the following results:

Group	**Absolute Range (ng/ml)**	**n**
Normal dogs	nd - 21	26
nd = non detectable		

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 canine prolactin. The reference ranges should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY

The lower detection limit for canine prolactin was 0.4 ng/ml.

SPECIFICITY

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

REPRODUCIBILITY

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 12 pairs of wells in a single run for Intra-Assay precision and the Inter-Assay precision was calculated from the results of 10 different runs of three samples:

Canine prolactin

Intra-Assay		
Sample No	Mean $\bar{x}$ (pg/ml)	CV (%)
1	6.8	7.1
2	29	6.0
3	50	7.4

Inter-Assay		
Sample No	Mean $\bar{x}$ (pg/ml)	CV (%)
1	8.8	9.2
2	15	6.9
3	32	5.5

RECOVERY

Three spiking solutions were prepared using the Sample Diluent, to represent the 600, 800 and 1000 ng/ml respectively. A 50 µl aliquot of each solution (A, B, C) was spiked into 950 µl aliquots of two different patient 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 canine ELISA procedure.

Sample	Diluted Solution	measured Concentration	expected Concentration	Recovery [%]
		[ng/ml]	[ng/ml]	
1	-	8.8	-	-
	A	35.3	38.4	92
	B	48.3	48.4	100
	C	56.2	58.4	96
2	-	6.1	-	-
	A	33.7	35.8	94
	B	49.7	45.8	109
	C	53.1	55.8	95

LINEARITY

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

Sample	Dilution Factor	measured Concentration	expected Concentration	Recovery [%]
		[ng/ml]	[ng/ml]	
1	8 in 8 4 in 8 2 in 8 1 in 8	54.9 24.4 12.8 6.6	- 27.5 13.7 6.9	- 89 93 96
2	8 in 8 4 in 8 2 in 8 1 in 8	54.2 27.8 15.4 7	- 27.1 13.6 6.8	- 103 113 103

LIMITATIONS OF PROCEDURE

The Prolactin canine ELISA has no "high-dose hook" effect, even with samples containing more than 300 ng/ml of canine prolactin. However, this effect is characteristic of immunometric assays. Samples expected to contain canine prolactin concentrations greater than the highest calibrator (80 ng/ml) should be diluted with the Canine Prolactin Calibrator/Sample Diluent.

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SHORT INSTRUCTION

(all sample sizes given in µl)

MP Well / Steps		0	1	2	3	4	5	6	Sample
	ng/ml	0	2.5	5	10	20	40	80	
	Solution								
Pipet	Calibrator	25	25	25	25	25	25	25	-
Pipet	Sample	-	-	-	-	-	-	-	25
Pipet	Canine Prolactin Sample Buffer	50	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 Canine Prolactin Ab	200	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	200
Incubate for 30 min at RT in the dark									
Pipet	Stop Solution	50	50	50	50	50	50	50	50
Read at λ = 450 nm									

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	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!