Canine Prolactin ELISA

For the quantitative determination of canine prolactin in serum samples.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 55-PRLCA-E01
Size: 96 wells
Version: 03-02/17 (190121) - ALPCO March 11, 2019
1. Introduction

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

1.2 Summary and Explanation
Canine prolactin (cPRL) is a single-chain polypeptide hormone of the canine anterior pituitary with a molecular mass of approximately 22,000 Da. 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 canine prolactin from the pituitary is inhibited by hypothalamic prolactin-inhibitory factor (PIF). Although dopamine was long thought to be this PIF molecule, today research suggests that there is a special peptide with prolactin-inhibiting activities. Studies demonstrate that the release of prolactin is certainly stimulated by different peptides, particularly thyrotropin releasing hormone (TRH) and vasoactive 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 canine prolactin.

The most important role of prolactin is stimulation of mammary gland growth and lactation. Research shows that 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 interestrus interval in female canines.

2. Principle of the Assay
The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of canine prolactin in serum. The microplate is coated with a monoclonal antibody specific for canine prolactin.

Calibators and samples are pipetted into the antibody coated microplate. During a 2 hour incubation, endogenous canine prolactin in the sample binds to the antibodies fixed on the inner surface of the wells. Non-reactive sample components are removed by a washing step. Afterwards, a polyclonal horseradish peroxidase-labeled antibody, directed against another epitope of the Prolactin molecule, is added. During a 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′-tetramethylbenzidine), is added to all wells. During a 30 minute 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.
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 samples 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 gloves when handling samples and reagents. Microbial contamination of reagents or samples 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 microtiter plate 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 be 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 national biohazard safety guidelines or regulations.
18. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available on ALPCO’s website or directly upon request.

4. Reagents

4.1 Reagents Provided

1. **Microtiter plate**, 12 x 8 (break apart) strips with 96 wells, ready to use; coated with a monoclonal anti-canine prolactin antibody

2. **Canine Prolactin Master Calibrator**, 1 vial, 80 ng, lyophilized; in serum/buffer matrix containing highly purified canine prolactin; for reconstitution see “Reagent preparation”
3. **Canine Prolactin Calibrator/Sample Diluent**, 1 vial, 6 mL, ready to use; canine prolactin free

4. **Enzyme Conjugate**, 1 vial, 22 mL, red, ready to use; containing horseradish peroxidase-labeled polyclonal anti-canine prolactin antibody

5. **Canine 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'-tetramethylbenzidine in buffered peroxide solution

7. **Stop Solution**, 1 vial, 7 mL, ready to use; contains 2M hydrochloric acid

8. **Wash Buffer Concentrate**, 1 vial, 50 mL, 10 x concentrated; see “Reagent preparation”

### 4.2 Materials required, but not provided

- Microplate reader capable of endpoint measurements at 450 nm (optional reference filter in the range of 600 - 690 nm)
- Vortex mixer
- Microplate mixer operating at more than 600 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
- Timer

### 4.3 Reagent Preparation

**Calibrators**: Reconstitute the lyophilized Canine Prolactin Master Calibrator with **1 mL distilled water** 30 minutes before use (end concentration of 80 ng/mL). Complete a serial dilution with Calibrator/Sample Diluent to make calibrators with the following concentrations: 80, 40, 20, 10, 5 and 2.5 ng/mL.

**Wash Buffer**: Dilute with 450 mL distilled 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°C to 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 Calibrators refrigerated, they are 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. **Sample**

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°C to 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 when calculating the results.

6. **Assay Procedure**

6.1 **General Remarks**

- Do not interchange components of different lots.
- All components should be at room temperature (21 °C to 26 °C) before use.
- All components of the test kits supplied as a concentrate, should be diluted to their final concentration at least 30 minutes prior to use. Mix well but prevent foam from forming.
- Use a disposable-tip micropipette to dispense serum samples. Pipet directly into the bottom of the wells. Change the tip between samples to avoid carryover contamination.

6.2 **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).

2. Pipet 25 µL of each calibrator and patient sample into their respective wells.

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

4. Rotate for 2 hours at room temperature (21°C to 26 °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 tapping the microplate carefully.
6. Add 200 µL of Enzyme Conjugate to all wells.

7. Shake again for 1 hour at room temperature (21 °C to 26 °C) on a plate mixer (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 tapping 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.

6.3 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.

6.4 Calibration Curve
Please refer to the QC certificate for lot specific calibration curve data.

7. Performance Characteristics

7.1 Analytical Sensitivity
The lower detection limit for canine prolactin is 0.4 ng/mL.

7.2 Analytical Specificity
The antibodies in the Canine Prolactin ELISA procedure are highly specific for canine prolactin. Detectable cross-reactivities to other hormones that may be present in serum samples are not known.

7.3 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:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8</td>
<td>7.1</td>
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<tr>
<td>2</td>
<td>29</td>
<td>6.0</td>
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<tr>
<td>3</td>
<td>50</td>
<td>7.4</td>
</tr>
<tr>
<td>Sample</td>
<td>Mean (pg/mL)</td>
<td>CV (%)</td>
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<tr>
<td>--------</td>
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<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>8.8</td>
<td>9.2</td>
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<tr>
<td>2</td>
<td>15</td>
<td>6.9</td>
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<tr>
<td>3</td>
<td>32</td>
<td>5.5</td>
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</table>

### 7.4 Recovery

Three spiking solutions were prepared using the Sample Diluent, to represent the 600, 800 and 1000 ng/mL prolactin, respectively. A 50 µL aliquot of each solution (A, B, C) was spiked into 950 µL aliquots of two different 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 Canine Prolactin ELISA kit.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diluted Solution</th>
<th>Measured Concentration [ng/mL]</th>
<th>Expected Concentration [ng/mL]</th>
<th>Recovery [%]</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>8.8</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>A</td>
<td>35.3</td>
<td>38.4</td>
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<tr>
<td></td>
<td>B</td>
<td>48.3</td>
<td>48.4</td>
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<tr>
<td></td>
<td>C</td>
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<td>2</td>
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<tr>
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<td>B</td>
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<tr>
<td></td>
<td>C</td>
<td>53.1</td>
<td>55.8</td>
<td>95</td>
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</table>

### 7.5 Linearity

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>Measured Concentration [ng/mL]</th>
<th>Expected Concentration [ng/mL]</th>
<th>Recovery [%]</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>8 in 8</td>
<td>54.9</td>
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<td></td>
<td>4 in 8</td>
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<td>2 in 8</td>
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<tr>
<td></td>
<td>1 in 8</td>
<td>7</td>
<td>6.8</td>
<td>103</td>
</tr>
</tbody>
</table>

### 8. Limitations of the Procedure

The Canine Prolactin 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.
9. References


10. Short Instruction

(all sample sizes given in µl)

<table>
<thead>
<tr>
<th>Steps</th>
<th>MP Well</th>
<th>ng/ml</th>
<th>0 (A)</th>
<th>1 (B)</th>
<th>2 (C)</th>
<th>3 (D)</th>
<th>4 (E)</th>
<th>5 (F)</th>
<th>6 (G)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solution</td>
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<tr>
<td>Pipet</td>
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<td>25</td>
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<tr>
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<tr>
<td>Incubate for 2 hours at RT on a shaker</td>
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<td>Decant Wash 4x with 300 µl of buffered wash solution</td>
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<tr>
<td>Decant Wash 4x with 300 µl of buffered wash solution</td>
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<td>Pipet</td>
<td>Substrate Solution</td>
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<td>Incubate for 30 min at RT in the dark</td>
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