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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 RD491488200R Canine Procalcitonin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of canine procalcitonin.

Features

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures canine procalcitonin in serum and urine
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2–8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.
3. INTRODUCTION

Procalcitonin (PCT), a polypeptide with a molecular mass of about 13 kDa, is the precursor of calcitonin. PCT is constitutively produced in the C cells of the thyroid gland and does not exhibit hormone activity [1]. It undergoes successive cleavages to form three distinct molecules; calcitonin, katacalcin and an N-terminal fragment called aminoprocalcitonin [2]. PCT belongs to a group of related proteins called the CAPA (CGRP – Amylin – Pro-calcitonin – Adrenomedullin) protein family [3]. Synthesis of PCT is regulated by the CALC-I gene [4]. Circulating levels of PCT in healthy human individuals was found to be very low (depend on source < 0.1 ng/ml or < 0.5 ng/ml) [3, 4].

PCT is rapidly produced and released to peripheral circulation in response to endotoxin and pro-inflammatory cytokines, such as IL-1β and TNF-α [1]. During microbial infection, there is an increase of CALC-I gene expression which causes a release of PCT from all parenchymal tissues and differentiated cell types throughout the body, including the liver and peripheral blood mononuclear cells [5].

PCT is markedly elevated within 2 to 4 hours in severe forms of systemic inflammation or in bacterial infections, and the level persists until recovery. The biological half-life of PCT is 22 to 26 hours, an advantageous time point compared with CRP and other acute-phase reactants [6]. Because up-regulation of PCT is attenuated by INF-γ, a cytokine released in response to viral infections, PCT is more specific for bacterial infections and may help to distinguish bacterial infections from viral illnesses [7].

It should be noted that PCT is also elevated in noninfectious conditions such as trauma, surgery, cardiogenic shock, burns, heat stroke, acute respiratory distress syndrome, infected necrosis after acute pancreatitis, and rejection after transplantation [5].

PCT is used as an early biomarker for the diagnosis of sepsis, severe sepsis and septic shock. PCT has also proved to be useful in guiding antibiotic therapy. This approach was mainly evaluated in patients with respiratory tract infections; however, it can also be used in critically ill patients with sepsis or severe sepsis of various origins [8].

Severe bacterial infections can result in marked morbidity and death in veterinary patients, with 50–70% of dogs with sepsis succumbing to their disease. Early diagnosis of infection is essential for the appropriate management of sepsis, as it allows rapid administration of antibiotics resulting in improved outcomes [9]. Although PCT mRNA expression from nonthyroidal tissue has been demonstrated in dogs with inflammation, sepsis and SIRS [3, 10], very little is known about serum PCT concentration in dogs due to the lack of a validated canine assay [9].

This ELISA assay is intended for the research of canine procalcitonin (PCT).

Areas of investigation:
Immune Response, Infection and Inflammation
(Sepsis, SIRS, etc)
4. TEST PRINCIPLE

In the BioVendor Canine Procalcitonin ELISA, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-canine procalcitonin antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-canine procalcitonin antibody is added and incubated with the captured procalcitonin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of canine procalcitonin. A standard curve is constructed by plotting absorbance values against procalcitonin concentrations of standards and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of animal origin. These materials should be handled as potentially infectious.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
• Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
• Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

7. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Coated Microtiter Strips</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Biotin Labelled Antibody</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Biotin-Ab Diluent</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>20 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Substrate Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Product Data Sheet + Certificate of Analysis</td>
<td>–</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

8. MATERIAL REQUIRED BUT NOT SUPPLIED

• Deionized (distilled) water
• Test tubes for diluting samples
• Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
• Precision pipettes to deliver 5–1000 μl with disposable tips
• Multichannel pipette to deliver 100 μl with disposable tips
• Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
• Vortex mixer
• Orbital microplate shaker capable of approximately 300 rpm
• Microplate washer (optional). [Manual washing is possible but not preferable.]
• Microplate reader with 450±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550–650 nm)
• Software package facilitating data generation and analysis (optional)
9. PREPARATION OF REAGENTS

- All reagents need to be brought to room temperature prior to use
- Always prepare only the appropriate quantity of reagents for your test
- Do not use components after the expiration date marked on their label

- Assay reagents supplied ready to use:

  Antibody Coated Microtiter Strips
  Stability and storage:
  Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2–8° C and protected from the moisture.

  Streptavidin-HRP Conjugate
  Biotin-Ab Diluent
  Dilution Buffer
  Substrate Solution
  Stop Solution
  Stability and storage:
  Opened reagents are stable 3 months when stored at 2–8° C.

- Assay reagents supplied concentrated or lyophilized:

  Canine Procalcitonin Master Standard
  Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
  Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the canine procalcitonin in the stock solution is 800 pg/ml.

Prepare set of standards using Dilution Buffer as follows:

<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Dilution Buffer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>–</td>
<td>800 pg/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>400 pg/ml</td>
</tr>
<tr>
<td>250 µl of 400 pg/ml</td>
<td>250 µl</td>
<td>200 pg/ml</td>
</tr>
<tr>
<td>250 µl of 200 pg/ml</td>
<td>250 µl</td>
<td>100 pg/ml</td>
</tr>
<tr>
<td>250 µl of 100 pg/ml</td>
<td>250 µl</td>
<td>50 pg/ml</td>
</tr>
<tr>
<td>250 µl of 50 pg/ml</td>
<td>250 µl</td>
<td>25 pg/ml</td>
</tr>
<tr>
<td>250 µl of 25 pg/ml</td>
<td>250 µl</td>
<td>12.5 pg/ml</td>
</tr>
</tbody>
</table>
Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Master Standard and diluted standard solutions.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute Biotin Labelled Antibody Concentrate (100x) with Biotin-Ab Diluent (e.g. 10 μl of Biotin Labelled Antibody Concentrate + 990 μl of Biotin-Ab Diluent for 8 wells).

Stability and storage:
Do not store diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare the 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2–8° C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2–8° C.

10. PREPARATION OF SAMPLES

The kit measures canine procalcitonin in serum and urine.

Samples can be assayed immediately after collection, or after a long-term storage. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

An appropriate dilution should be assessed by the researcher in advance to batch measurement.

Recommended starting dilution for canine serum/urine is 5x.

Dilute serum/urine samples 5x with the Dilution Buffer just prior to the assay as follows:
Add 50 μl of sample into 200 μl of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet 100 µl of diluted Standards, Dilution Buffer (= Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µl of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µl of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for 15 minutes at room temperature. The incubation time may be extended [up to 25 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 µl of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550–650 nm). Subtract readings at 630 nm (550–650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

Note 1: If some samples and standard/s have absorbance above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine canine procalcitonin concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.
Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

<table>
<thead>
<tr>
<th></th>
<th>strip 1+2</th>
<th>strip 3+4</th>
<th>strip 5+6</th>
<th>strip 7+8</th>
<th>strip 9+10</th>
<th>strip 11+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 800</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 25</td>
<td>Sample 33</td>
</tr>
<tr>
<td>B</td>
<td>Standard 400</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>C</td>
<td>Standard 200</td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>D</td>
<td>Standard 100</td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
<td>Sample 28</td>
<td>Sample 36</td>
</tr>
<tr>
<td>E</td>
<td>Standard 50</td>
<td>Sample 5</td>
<td>Sample 13</td>
<td>Sample 21</td>
<td>Sample 29</td>
<td>Sample 37</td>
</tr>
<tr>
<td>F</td>
<td>Standard 25</td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</td>
</tr>
<tr>
<td>G</td>
<td>Standard 12.5</td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
<td>Sample 39</td>
</tr>
<tr>
<td>H</td>
<td>Blank</td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
<td>Sample 40</td>
</tr>
</tbody>
</table>

Figure 1: Example of a work sheet.
12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of procalcitonin (pg/ml) in canine samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because they have been diluted prior to the assay, e.g. 100 pg/ml (from standard curve) x 5 (dilution factor) = 500 pg/ml.

![Canine Procalcitonin ELISA Standard Curve](image)

*Figure 2: Typical standard curve for Canine Procalcitonin ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Canine Procalcitonin ELISA are presented in this chapter.

- **Sensitivity**
  Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3 \times SD_{blank}$) is calculated from the real canine procalcitonin values in wells and is 3.6 pg/ml.
  * Dilution Buffer is pipetted into blank wells.

- **Limit of Assay**
  Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**
  Less than 10% cross-reactivity (CR) was observed with recombinant porcine procalcitonin at concentration 1 ng/ml.
  Less than 1% cross-reactivity (CR) was observed with recombinant monkey (*Macaca mulatta*) procalcitonin at concentration 1 ng/ml.
  No significant CR was observed for tested serum samples of other mammalian species. For details please contact us at info@biovendor.com.

Presented results are multiplied by respective dilution factor

- **Precision**
  Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>528.5</td>
<td>19.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Serum 2</td>
<td>242.0</td>
<td>11.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>511.6</td>
<td>34.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Serum 2</td>
<td>222.3</td>
<td>16.6</td>
<td>7.5</td>
</tr>
</tbody>
</table>
• **Spiking Recovery**

Canine serum and urine samples were spiked with different amounts of canine procalcitonin and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>140.0</td>
<td>265.0</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>262.4</td>
<td>390.0</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>381.2</td>
<td>640.0</td>
<td>93.8</td>
</tr>
<tr>
<td>Serum 2</td>
<td>217.6</td>
<td>342.6</td>
<td>88.8</td>
</tr>
<tr>
<td></td>
<td>304.4</td>
<td>467.6</td>
<td>94.8</td>
</tr>
<tr>
<td></td>
<td>443.2</td>
<td>717.6</td>
<td>95.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 1</td>
<td>128.4</td>
<td>253.4</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>241.6</td>
<td>378.4</td>
<td>93.7</td>
</tr>
<tr>
<td></td>
<td>354.4</td>
<td>628.4</td>
<td>95.3</td>
</tr>
</tbody>
</table>

• **Linearity**

Canine serum and urine samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>-</td>
<td>377.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>185.2</td>
<td>188.6</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>93.2</td>
<td>94.3</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>47.2</td>
<td>47.2</td>
<td>100.1</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>480.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>246.4</td>
<td>240.2</td>
<td>102.6</td>
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<tr>
<td></td>
<td>4x</td>
<td>120.4</td>
<td>120.1</td>
<td>100.2</td>
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<tr>
<td></td>
<td>8x</td>
<td>60.4</td>
<td>60.1</td>
<td>100.6</td>
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</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 1</td>
<td>-</td>
<td>504.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>232.4</td>
<td>252.0</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>116.4</td>
<td>126.0</td>
<td>92.4</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>62.0</td>
<td>63.0</td>
<td>98.4</td>
</tr>
</tbody>
</table>
14. DEFINITION OF THE STANDARD

In this assay the recombinant protein (E. coli) is used as the standard. The recombinant procalcitonin is a 12.7 kDa protein consisting of 105 amino acid residues of the canine procalcitonin and 10 extra aminoacids.

15. PRELIMINARY POPULATION DATA

- Reference range
Mean concentration of canine procalcitonin in serum samples from 24 healthy donors (beagle dogs) was $281 \pm 115$ pg/ml (median 260 pg/ml). However, the data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for canine procalcitonin levels with the assay.

16. TROUBLESHOOTING AND FAQs

- **Weak signal in all wells**
  Possible explanations:
  - Omission of a reagent or a step
  - Improper preparation or storage of a reagent
  - Assay performed before reagents were allowed to come to room temperature
  - Improper wavelength when reading absorbance

- **High signal and background in all wells**
  Possible explanations:
  - Improper or inadequate washing
  - Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
  - Incubation temperature over 30°C

- **High coefficient of variation (CV)**
  Possible explanation:
  - Improper or inadequate washing
  - Improper mixing Standards or samples
17. REFERENCES

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systemic infections in chronic kidney disease or renal transplant patients. *Int Urol Nephrol*;
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AM, Bienzle D. Investigation of a commercial ELISA for the detection of canine


For more references on this product see our WebPages at www.biovendor.com
## 18. EXPLANATION OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
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<td>Attention, see instructions for use</td>
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<tr>
<td>🦠</td>
<td>Potential biological hazard</td>
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<tr>
<td>⌛️</td>
<td>Expiry date</td>
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<tr>
<td>🌡️  8°C</td>
<td>Storage conditions</td>
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<td>Name and registered office of the manufacturer</td>
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Assay Procedure Summary

1. Reconstitute Biotin Labelled Antibody and Master Standard, prepare set of standards
2. Add standards, and samples 100 μl
3. Incubate at RT for 1 hour / 300 rpm
4. Prepare Wash Solution
5. Wash 3x
6. Add Biotin Labelled Antibody solution 100 μl
7. Incubate at RT for 1 hour / 300 rpm
8. Wash 3x
9. Add Streptavidin-HRP Conjugate 100 μl
10. Incubate at RT for 30 min / 300 rpm
11. Wash 3x
12. Add Substrate Solution 100 μl
13. Incubate at RT for 15 min
14. Add Stop Solution 100 μl
15. Read absorbance and calculate results

- Dilute samples: serum and urine 5x