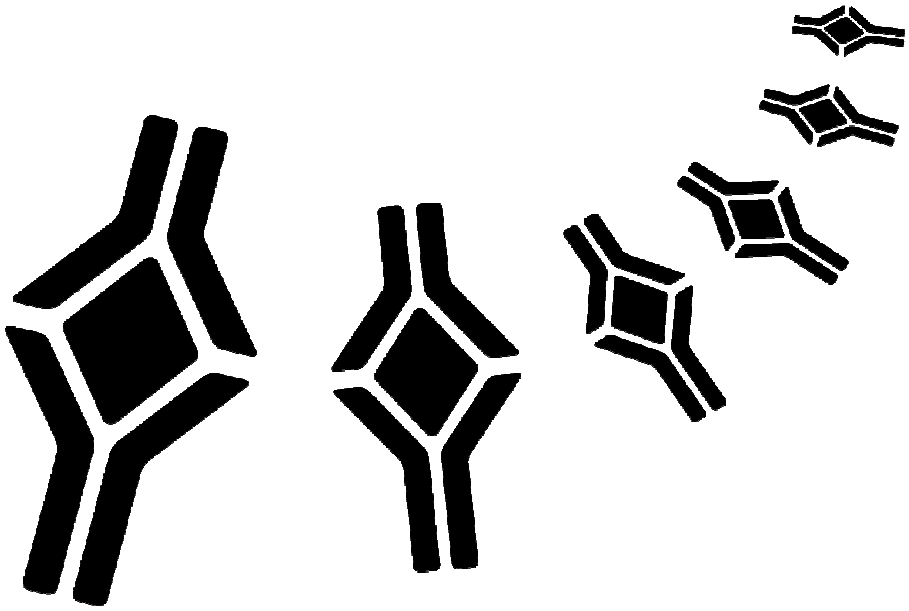
BioVendor
Research
and Diagnostic Products



Canine C-Reactive Protein ELISA

Product Data Sheet

Cat. No.: RH931CRP01DCR

For Research Use Only

VERSION 51 020611 46

CONTENTS

» **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 C-reactive protein assay is intended for the detection and quantification of canine C-reactive protein (CRP) in dog serum. C-reactive protein is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, or tissue trauma. Quantification of CRP is useful in determining inflammatory conditions difficult to diagnose and to monitor the patients' response to treatment.

2. PRINCIPLE OF ASSAY

Canine sera for testing are diluted to 1:500 and allowed to react with pneumococcal C-polysaccharide coated on specially treated microwells. After appropriate incubation, the wells are washed to remove unreacted serum proteins, and an enzyme-labeled goat anti-dog CRP (conjugate) is then added to react with and tag the antigen-antibody complexes. Following another incubation period, the wells are again washed to remove unreacted conjugate. A urea peroxide substrate with TMB as chromogen is added to start color development. Development of a blue color indicates a positive reaction while negative reactions appear colorless or with a trace of blue. The reaction is interrupted with a stop solution that turns the blue positive reactions to yellow. Negative reactions remain colorless or with a hint of yellow. Color intensity (absorbance) is read at a wavelength of 450 nm on a spectrophotometer or ELISA reader. Semi-quantification of absorbance can be accomplished by the use of a standard curve generated by measuring four-fold dilutions of the standard provided.

3. REAGENTS AND MATERIALS SUPPLIED

This kit supplies sufficient materials for 96 determinations.

1. **CRP ELISA microplate**
   96-well plate containing pneumococcal C-polysaccharide and packaged with desiccant, ready to use.
2. **Conjugate (100x), 0.13 mL**
   Concentrated affinity-purified horseradish peroxidase (HRP)-labeled goat anti-dog CRP-IgG with stabilizers and a preservative. Protect from light.
3. **CRP Standard, 10X, 0.25 mL,**
   Dog serum with elevated CRP concentration. Dilute 1:10 for standard 1, then serially dilute three-fold. Contains proclin 150 as preservative.
4. **Wash Buffer, 1 packet**
   Tris with Tween 20, pH 7.4 and 0.05% Tween 20 when reconstituted to 1L with distilled water.
5. **TMB Substrate, 12 mL**
   A solution containing urea peroxide and 3,3', 5,5'-tetramethylbenzidine (TMB) supplied in a protective opaque bottle. Ready to use. Protect from light. Non-carcinogenic.
6. **Stop Solution, 12 mL**
   Diluted phosphoric acid, ready to use.

4. WARNING

1. DO NOT INTERCHANGE COMPONENTS BETWEEN KITS AND DIFFERENT LOTS OF THE SAME TEST.
2. The control sera have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, however, these reagents, as well as the serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
3. The coated microwells are prepared with inactivated antigens. However, they should be considered potentially infectious and handled accordingly.

Storage and Handling

Kit components should be stored at 2-8°C. Bring them to room temperature (20-25°C) before opening bottles and plate pouches. Diluted conjugate remaining after use should be discarded. TMB substrate and stop solution are also stable at room temperature.

5. SPECIMENS ABLE TO BE USED FOR THIS ASSAY

- Serum
- Plasma
- Urine
- Culture Supernatant
- Tissue Extracts
- Synovial Fluid

6. BLOOD COLLECTION AND PREPARATION

Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. If testing is to be delayed longer than 5 days, freezing the sample at -20°C or colder is recommended.
See Assay Procedure Step 3 for dilution instructions

7. PREPARATION OF OTHER SPECIMEN TYPES

Samples other than blood (plasma or serum) should be used at a higher concentration. It is recommended to begin diluting the sample at 1:2 and increasing the dilution factor to values the user sees fit.

8. ASSAY PROCEDURE

PROCEDURAL NOTES

1. **IMPORTANT:**
   Bring kit components to room temperature (20-25°C) before opening bottles and plate pouches. Allow at least 30 minutes for this process.

2. MULTI-SAMPLE DILUTION:
   For assays on many samples, it is advisable to pre-dilute samples onto microtiter dilution wells before using a multichannel pipetter to transfer it onto the coated wells. This will ensure the least variable incubation period in samples in multiple test strips.

3. DUPLICATE/TRIPLICATE TESTING:
   It is highly recommended to run standards and unknown samples in duplicates or triplicates to ensure precision.

PROCEDURE

Materials Required But Not Supplied

1. Distilled or deionized (purified) water
2. Clean 250 or 500 mL wash bottle for wash buffer.
3. Test tubes or microtiter plate for preparing standard dilutions.
4. Precision pipette(s) (2 µL to 1000 µL) for making and delivering dilutions.
5. Adhesive cover for microplates.
6. ELISA reader equipped with a 450 nm filter. A program for data reduction would be helpful.

Precautions

1. Do not use components past expiration date.
2. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

IMPORTANT: Bring kit components to room temperature (20-25°C) before opening bottles and plate pouches. Allow at least 30 minutes for this process.

ASSAY PROCEDURE

1. Prepare wash buffer by adding 1 packet of powder to 1L of distilled water.
2. Prepare the standards as follows:
   - **Standard 1 = 40.0 µg/mL**: 1:10 dilution of standard provided.
   - **Standard 2 = 13.3 µg/mL**: dilute Standard 1 three-fold, e.g. 1 unit of standard 1 plus 2 units of wash buffer.
   - Standards 3 (4.4 µg/mL) and **standard 4 (1.5 µg/mL)** are prepared by serial three-fold dilutions following standard 2.

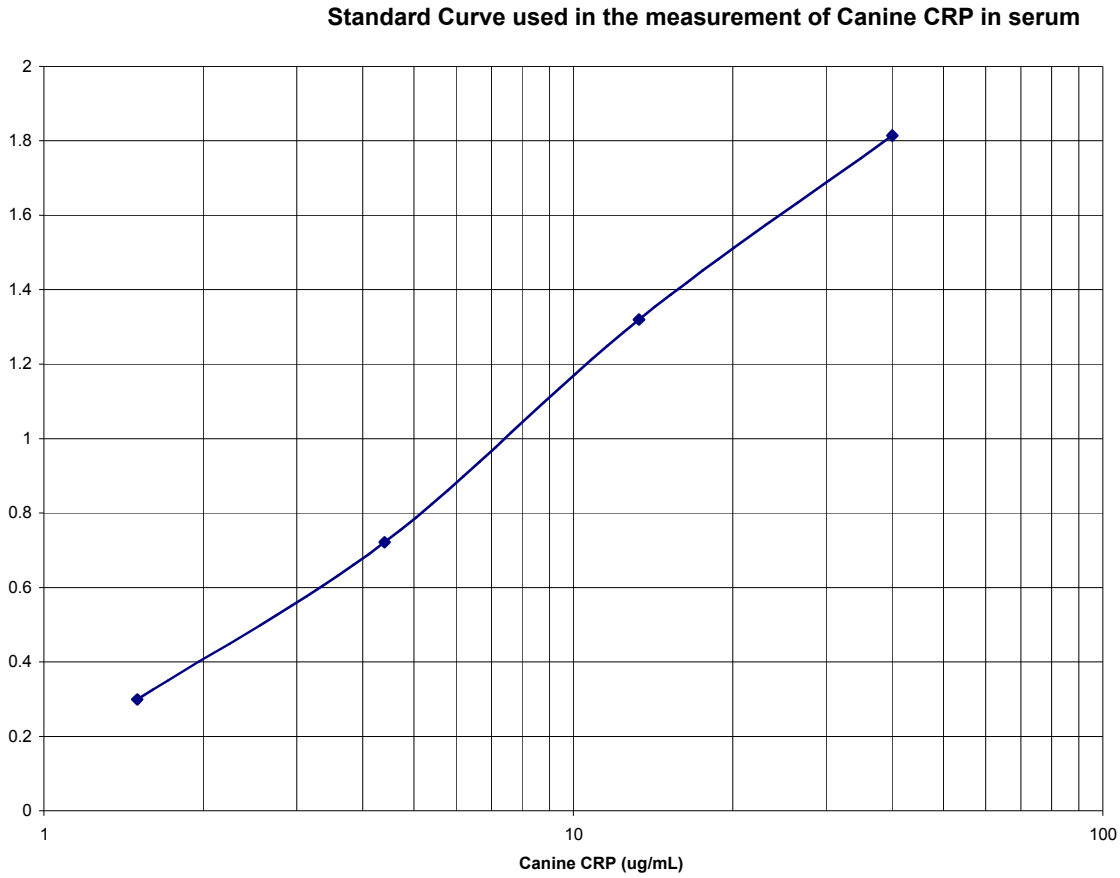
Please consider the following dilution scheme as a guide

Standard #	Concentration	Volume Transferred	Diluent Volume	Total Volume	Final Volume
					(after dilutions)
1	40.0 µg/mL	36 µL	324 µL	360 µL	240 µL
2	13.3 µg/mL	120 µL	240 µL	360 µL	240 µL
3	4.4 µg/mL	120 µL	240 µL	360 µL	240 µL
4	1.5 µg/mL	120 µL	240 µL	360 µL	360 µL

3. Dilute each serum sample 1:500 as follows: into a dilution vial, add 1mL of wash buffer. To this, add 2 µL of serum.
4. Add 100 µL to each well and incubate at ambient temperature for 30 minutes. Record the location for later reference.
5. Wash plates 3 - 4 times with a gentle stream of wash buffer from a wash bottle or a plate washer. Tap plates on a stack of absorbent paper towels to remove residual buffer.
6. Dilute stock conjugate (100x) to the desired working dilution (1x) with the Tris buffer, e.g. to 10 mL buffer, add 100 µL stock conjugate. This amount is sufficient for processing the whole plate. If only a part of the plate is being processed, the necessary aliquot has to be pipetted.
7. To each microwell, add 100 µL of conjugate.
8. Cover plate and incubate for 30 minutes at ambient temperature (20-25°C).
9. Wash plate as in step 5.
10. To each microwell, add 100 µL TMB/substrate solution and allow reaction to proceed at ambient temperature for 5-10 minutes. Cover to avoid direct lighr. A blue color indicates a positive reaction.
11. Stop reaction by adding 100 µL of Stop solution to each well. Reaction mixture turns from blue to yellow.

12. Read absorbance (OD) on a microplate reader equipped with a 450 nm filter. Construct standard curve and read off values for patient samples. Multiply values by 5 to get actual serum concentration.

9. RESULTS



Reference range: 3 – 25 µg/mL
Abnormal: over 25 µg/mL

10. LIMITATIONS

Lipemic sera may interfere with specific antibody reaction.

11. QUALITY CONTROL

Routinely run at least two controls each giving values at the top or bottom regions of the standard curve respectively.
An occasional prozone may be encountered in sera with high CRP values. In this situation, due to antigen excess, all the CRP available may not have reacted with the conjugate. Therefore, test at higher dilution, e.g. 1:1,000 or 1:2,000 to obtain more accurate results.

12. PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Inter-assay reproducibility (2 plate lots)

	CRP (µg/mL)	CV (%)
QC-High	40.0	2.7
QC-Low	1.3	1.8

Intra-assay reproducibility (n=12))

	CRP (µg/mL)	CV (%)
QC-High	40.0	3.5
QC-Low	1.3	3.0

SENSITIVITY
The canine CRP assay is designed to detect elevated levels of CRP. The following data was produced to generate data on the sensitivity of the assay and maybe useful in research applications where sensitivity parameters need to be defined.

Assay Sensitivity (n=11)

Sample	Mean [OD]	Standard Variation	Detection Limit [ng/ml]
1	0.056	0.007	3.6 ng/ml

CROSS REACTIVITY

Rat CRP	0/15	0%

13. REFERENCES

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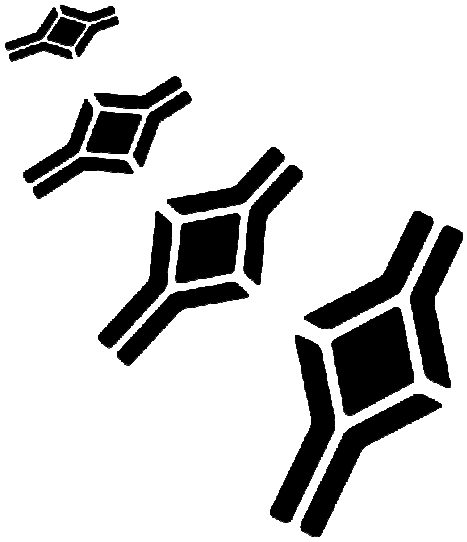

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