

**DRG<sup>®</sup> CRP (canine) ELISA (EIA-4694)**

Revised 21 March 2007 (Vers. 1.1)

**For Veterinary Use Only****THIS KIT IS INTENDED FOR RESEARCH USE ONLY.****NOT FOR USE IN DIAGNOSTIC PROCEDURES.****1 INTENDED USE**

The CRP (canine) ELISA is intended for the detection and quantification of canine C-reactive protein (CRP) in dog serum.

**For research use only.**

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 antibodies coated on specially treated micro-wells. 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.

- CRP ELISA microplate**  
96-well plate containing an affinity purified goat anti-dog CRP-IgG and packaged with desiccant, ready to use.
- Conjugate (100X)**, 0.12 mL  
Concentrated affinity purified horseradish peroxidase (HRP) labeled goat anti dog CRP-IgG with stabilizers and a preservative.  
*Protect from light.*
- 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.

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4. **Wash Buffer**, 1 packet  
Phosphate-buffered saline (PBS) with Tween 20, pH 7.4 and 0.05% Tween 20 when reconstituted to 1 L with distilled water.
5. **TMB Substrate Solution**, 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.

**5 STORAGE AND HANDLING**

Kit components should be stored at 2 °C – 8 °C. Bring them to room temperature (20 °C – 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.

**6 SPECIMEN COLLECTION**

Qualified personnel should collect blood samples using approved venipuncture techniques. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container.

Store at 2 °C – 8 °C. If testing is to be delayed longer than 5 days, freezing the sample at –20 °C or colder is recommended.

Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained 1-2 weeks later.

**7 MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Distilled or deionized (purified) water
2. Clean 250 mL 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.

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6. ELISA reader equipped with a 450 nm filter. A program for data reduction would be helpful.

**8 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 °C – 25 °C) before opening bottles and plate pouches. Allow at least 30 minutes for this process.

**9 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	18 µL	162 µL	180 µL	120 µL
2	13.3 µg/mL	60 µL	120 µL	180 µL	120 µL
3	4.4 µg/mL	60 µL	120 µL	180 µL	120 µL
4	1.5 µg/mL	60 µL	120 µL	180 µL	180 µL

3. Dilute each serum sample **1:500** as follows:  
 into a dilution vial, add 1 mL 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 PBS-T buffer.
7. To each microwell, add 100 µL of conjugate.

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8. Cover plate and **incubate for 30 minutes** at ambient temperature (20 °C -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 **10 minutes**. 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.

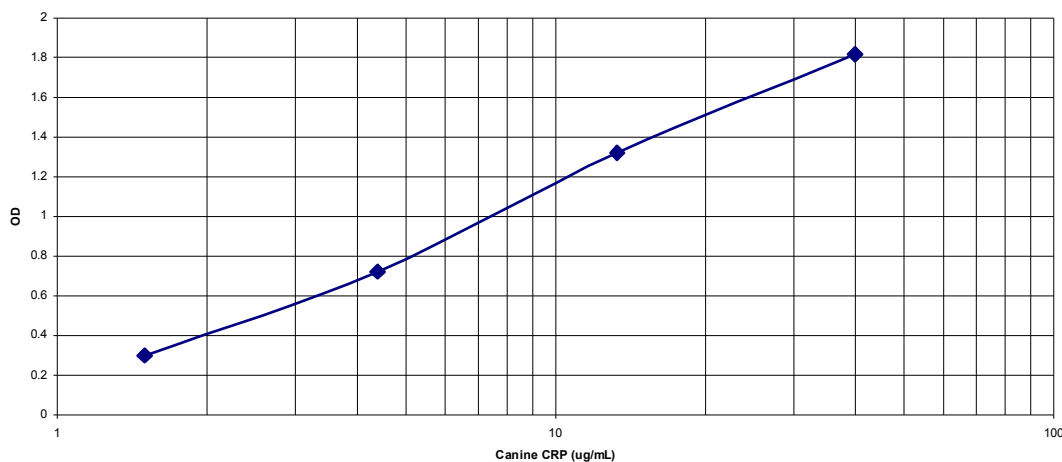
Multiply values by 5 to get actual serum concentration.

### 10 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.

### 11 REPORTING OF RESULTS

Standard Curve used in the measurement of Canine CRP in serum



Reference range: 3 – 25 µg/mL

Abnormal: over 25 µg/mL

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**12 LIMITATIONS**

Lipemic sera may interfere with specific antibody reaction.

**13 PERFORMANCE CHARACTERISTICS**

**13.1 Reproducibility**

Inter-assay reproducibility (2 plate lots)	
<b>µg/mL</b>	<b>CV (%)</b>
High (40.0)	2.7
Low (1.3)	1.8

Intra-assay reproducibility (tested 12 times)	
<b>µg/mL</b>	<b>CV (%)</b>
High (40.0)	3.5
Low (1.3)	3.0

**13.2 Sensitivity**

The CRP (canine) ELISA 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

**13.3 Cross reactivity**

Rat CRP            0/15            0%

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1. Burton, S.A., et. al. C-reactive protein concentration in dogs with inflammatory leukograms. *Am. J. Vet. Res.* 1994; 55:613 – 618.
2. Casals, C. et. al. Increase of C-reactive protein and decrease of surfactant protein A in surfactant after lung transplantation. *Am. J. Respir. Crit. Care. Med.* 1998; 157: 43 – 49.
3. Conner, J.G., et. al. Acute phase response in the dog following surgical trauma. *Res. Vet. Sci.* 1988; 45: 107 – 110.
4. Eckersall, P.D. et. al., An immunoturbidimetric assay for canine C-reactive protein. *Vet. Res. Commun.* 1991; 15: 17 – 24.
5. Lindback, S., et. al. The value of C-reactive protein as a marker of bacterial infection in patients with septicemia, endocarditis, and influenza. *Scand. J. Infect. Dis.* 1989; 21: 543 – 549.
6. Ndung'u, J.M. et. al. Elevation of the concentration of acute phase proteins in dogs infected with *Trypanosoma brucei*. *Acta. Trop.* 1991;49: 77 – 86.
7. Otabe, K. Physiological levels of C-reactive protein in normal canine sera. *Vet. Res. Commun.* 1998; 22: 77 – 85.
8. Rikihisa, Y. et. al. C-reactive protein and alpha1-acid glycoprotein levels in dogs infected with *Ehrlichia canis*. *J. Clin. Microbiol.* 1994; 32: 912 – 917.
9. Yamamoto, S. et. al. Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test. *Vet. Q.* 1994; 16: 74 – 77.
10. Yamashita, K., et. al. Canine acute phase response: relationship between serum cytokine activity and acute phase protein in dogs. *J. Vet. Med. Sci.* 1994; 56: 487 – 492.

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