

# DOG C-REACTIVE PROTEIN (CRP) ELISA TEST KIT

Life Diagnostics, Inc., Catalog Number: 2210-3

## Enzyme Immunoassay for the Quantitative Determination of Dog C-Reactive Protein (CRP) in Serum

### INTRODUCTION

CRP is an acute phase protein that is elevated in serum as a result of injury, infection, pregnancy or disease. Baseline levels of CRP in dogs have been reported in the range of 1-100  $\mu\text{g/ml}$  with elevations of approximately 10 fold during pregnancy and 23-95 fold during infection. Measurement of CRP provides a convenient marker of inflammation and disease.

### PRINCIPLE OF THE TEST

The dog CRP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses anti-dog CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-dog CRP antibodies for detection. The test sample is diluted and incubated in microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. CRP molecules are thereby sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of CRP is proportional to the optical density of the test sample.

### MATERIALS AND COMPONENTS

#### **Materials provided with the kit:**

- Anti-dog CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (3 vials, lyophilized), containing dog CRP
- 10x Diluent (25 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

#### **Materials required but not provided:**

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer.
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength with an optical density range of 0-4 OD
- Graph paper (PC graphing software is optional)

### STORAGE OF TEST KIT AND INSTRUMENTATION

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

### GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum or plasma samples should be diluted ~10,000 fold with 1x diluent in order to obtain values within the standard range.

### DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

### WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

### STANDARD PREPARATION

1. Add 1 ml of distilled or de-ionized water to one of the dog CRP standard vials and mix gently until dissolved. Use the reference standard within one hour of reconstitution and discard after use.
2. Label 8 polypropylene or glass tubes: 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0 ng/ml.
3. Prepare a 100 ng/ml working CRP standard as detailed on the standard vial label, by mixing the indicated volume of diluent and reconstituted standard in the tube labeled 100 ng/ml.
4. Dispense 400  $\mu\text{l}$  of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.125, 1.56, and 0 ng/ml.
5. Prepare a 50 ng/ml standard by diluting and mixing 400  $\mu\text{l}$  of the 100 ng/ml standard with 400  $\mu\text{l}$  of diluent in the tube labeled 50 ng/ml. Similarly prepare the 25, 12.5, 6.25, 3.125, and 1.56 ng/ml standards by serial dilution.

### SAMPLE PREPARATION

**General Note:** CRP is reportedly present in normal dog serum at concentrations ranging from 1 to 100  $\mu\text{g/ml}$ . In order to obtain values within the range of the standard curve we suggest that samples initially be diluted 10,000 fold using the following procedure for each sample to be tested:

1. Dispense 495  $\mu\text{l}$  of 1x diluent into two separate tubes.
2. Pipette and mix 5  $\mu\text{l}$  of the serum/plasma sample into the first tube. This provides a 100 fold diluted sample.

- Mix 5  $\mu\text{l}$  of the 100 fold diluted sample with the 495  $\mu\text{l}$  of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
- Repeat this procedure for each sample to be tested

### ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100  $\mu\text{l}$  of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
- Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
- Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (350  $\mu\text{l}$ /well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Add 100  $\mu\text{l}$  of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
- Wash as detailed in 4 to 5 above.
- Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
- Dispense 100  $\mu\text{l}$  of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100  $\mu\text{l}$  of Stop Solution to each well.
- Gently mix. It is important to make sure that all the blue color changes to yellow.
- Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

### CALCULATION OF RESULTS

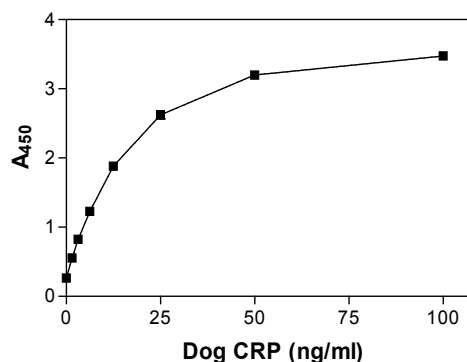
- Calculate the average absorbance values ( $A_{450}$ ) for each set of reference standards, and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of CRP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of CRP in the serum/plasma sample.
- If available, PC graphing software may be used for the above steps.
- If the  $OD_{450}$  values of samples fall outside the standard curve when tested at a dilution of 10,000. Samples should be diluted appropriately and re-tested.

### TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against CRP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CRP (ng/ml)	Absorbance (450 nm)
0	0.265
1.56	0.557
3.125	0.825
6.25	1.228
12.5	1.882
25	2.623
50	3.200
100	3.470

Typical Dog CRP Standard Curve



### LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

### ADDITIONAL INFORMATION

The concentration of dog CRP in the lyophilized standards provided with the kit was determined by reference to purified dog CRP prepared at Life Diagnostics Inc.