

DOG C-REACTIVE PROTEIN (CRP) ELISA

Life Diagnostics, Inc., Catalog Number: CRP-4

INTRODUCTION

CRP is an acute phase protein that is elevated in serum due to injury, infection, pregnancy or disease. Levels of CRP in dogs have been reported in the range of 1-200 $\mu\text{g/ml}$ ^{1,2} with elevations of approximately 10-fold during pregnancy and up to 100-fold during infection. Measurement of CRP provides a convenient biomarker of inflammation and disease in dogs.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified dog CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated dog CRP antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in CRP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If CRP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of CRP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- CRP antibody coated 96-well plate (12 x 8-well strips) **Store at -20°C**
- HRP Conjugate, 11 ml
- CRP stock (lyophilized, 3 vials)
- 20x Wash solution: CRPW50-20, 50 ml
- Diluent: YD50-1, 2 x 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

Store CRP antibody coated 96-well plate at -20°C. Store the remainder of the kit at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT

The diluent is provided ready to use. Do not use other dilution buffers.

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. The CRP stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved. Use within one hour of reconstitution.
2. Label 5 polypropylene or glass tubes as 10, 5, 2.5, 1.25 and 0.625 ng/ml.
3. In the tube labeled 10 ng/ml prepare the 10 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 μl of diluent into the tubes labeled 5, 2.5, 1.25 and 0.625 ng/ml.
5. Prepare the 5 ng/ml standard by mixing 250 μl of the 10 ng/ml standard with 250 μl of diluent in the tube labeled 5 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

CRP is present in dog serum at concentrations up to 200 $\mu\text{g/ml}$. Because of the wide range of concentrations, we suggest testing samples at dilutions of 1000-fold and 20,000-fold to obtain absorbance values within the mid-range of the standard curve. Dilutions may be obtained as follows:

1. Dispense 198 μl into one tube, 270 μl into a second tube, and 285 μl into a third tube.
2. Pipette and mix 2.0 μl of the serum/plasma sample into the tube containing 198 μl of 1x diluent. This provides a 100-fold dilution.
3. Mix 30 μl of the 100-fold diluted sample with the 270 μl of diluent in the second tube. This provides a 1000-fold dilution.
4. Mix 15 μl of the 1000-fold diluted sample with the 285 μl of diluent in the third tube. This provides a 20,000-fold dilution.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at -20°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

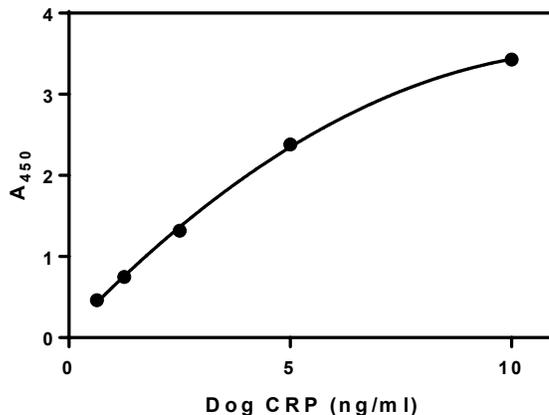
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model (we use a single site, total and nonspecific binding or second order polynomial models) and determine the concentration of the samples from the standard curve.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside or at the extremes of the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for 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)
10	3.428
5	2.381
2.5	1.317
1.25	0.748
0.625	0.463



ADDITIONAL INFORMATION

The concentration of CRP in the lyophilized standards provided with the kit was determined by reference to purified dog CRP (catalog no. 8101) prepared at Life Diagnostics, Inc.

REFERENCES

1. Nakamura M., et. al. C-reactive protein concentration in dogs with various diseases. *J Vet Med Sci.* 70(2):127-131 (2008)
2. Shida T., et.al. Characteristics of C-reactive protein (CRP), a1-acid glycoprotein (AAG) and serum amyloid A (SAA) in dogs and cats with malignant cancer. *Intern J Appl Res Vet Med.* 9(4):376-381 (2011)

Rev 05122021

***PROTOCOL AND MATERIALS MODIFIED 02/21/2021**

For technical assistance please email us at
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