

CAT C-REACTIVE PROTEIN (CRP) ELISA

Life Diagnostics, Inc., Catalog Number: CRP-8

INTRODUCTION

CRP is an acute phase protein that is elevated in the serum of many mammalian species because of injury, infection, or disease. CRP levels can increase several hundred-fold in humans, monkeys and dogs. In cats, CRP is considered a more modest acute phase reactant. Levels have been reported to increase 2-fold.^{1,2} In studies at Life Diagnostics, using our recently developed cat CRP SPARCL™ kit, we found levels ranging from 50 µg/ml to > 1 mg/ml in a bank of serum samples from healthy and sick cats, suggesting that CRP might be a more responsive acute phase biomarker in cats than previously thought.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified cat CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated cat 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, and 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)
- HRP Conjugate, 11 ml
- CRP stock (lyophilized, 3 vials)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 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 the kit at 2-8°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 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 volume of the 10x stock with nine 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. Reconstitute the CRP stock as described on the vial label. The reconstituted stock is stable for one day at 4°C but should be discarded thereafter.
2. Label 7 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml.
3. Prepare a 50 ng/ml CRP standard as detailed on the stock vial in the tube labeled 50 ng/ml.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml.
5. Prepare a 25 ng/ml standard by diluting and mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.
6. Similarly prepare the remaining standards by serial dilution.

SAMPLE PREPARATION

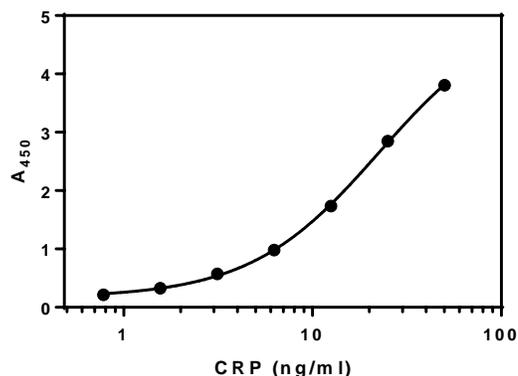
To obtain values within the range of the standard curve we suggest that samples be diluted 10,000-fold using the following procedure for each sample to be tested.

1. Dispense 98 µl and 497.5 µl of 1x diluent into two separate tubes.
2. Pipette and mix 2.0 µl of the serum/plasma sample into the tube containing 98 µl of 1x diluent. This provides a 50-fold dilution.
3. Mix 2.50 µl of the 50-fold diluted sample with the 497.5 µl of 1x diluent in the second tube. This provides a 10,000-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 2-8°C for future use.

2. Dispense 100 μ l 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 μ l/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ l 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 μ l 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 μ l 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.



REFERENCES

1. Leal RO., et.al. Monitoring acute phase proteins in retrovirus infected cats undergoing feline interferon-w therapy. J Small Animal Practice. 55:39-45 (2014)
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 121117

For technical assistance please email us at
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CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus \log_{10} of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = \log_{10} concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
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 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)
50	3.808
25	2.846
12.5	1.735
6.25	0.981
3.13	0.573
1.56	0.329
0.78	0.214