PIG C-REACTIVE PROTEIN (CRP) ELISA
Life Diagnostics, Inc., Catalog Number: CRP-9

INTRODUCTION

CRP is an acute phase protein that is elevated in serum because of injury, infection, or disease. Baseline levels of CRP in pigs range from 5-30 μg/ml. Levels may increase to 500 μg/ml during the acute phase response.[1-4] Measurement of CRP provides a convenient marker of inflammation and disease.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified pig CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated pig CRP antibodies for detection. Standards and diluted samples are incubated in wells of a 96-well plate for 45 minutes. The wells are subsequently washed. HRP conjugate, prepared from a concentrated stock 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:

- Anti-CRP coated 96-well plate (12 x 8-well strips)
- HRP Conjugate Stock, 1 vial. **Store at -20°C**
- CRP stock (lyophilized, 2 vials). **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: CSD50-1, 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
- Microcentrifuge and 15 ml test 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

The lyophilized standards and conjugate stock must be stored at or below -20°C when received. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. Test kits will remain stable for six months from the date.

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.

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. Use the standard within 1 hour of reconstitution and discard after use.
2. Label eight microcentrifuge tubes as 150, 75, 37.5, 18.75, 9.38, 4.67, 2.34 and 0 ng/ml.
3. Prepare a 150 ng/ml standard as detailed on the standard vial label, by mixing the indicated volume of diluent and reconstituted standard in the tube labeled 150 ng/ml.
4. Dispense 250 μl of diluent into the tubes labeled 75, 37.5, 18.75, 9.38, 4.67, 2.34 and 0 ng/ml.
5. Prepare a 75 ng/ml standard by diluting and mixing 250 μl of the 150 ng/ml standard with 250 μl of diluent in the tube labeled 75 ng/ml. Similarly prepare the 37.5 to 2.34 ng/ml standards by serial dilution.

SAMPLE PREPARATION

CRP is present in normal pig serum at concentrations of 5-30 μg/ml. Levels can exceed 500 μg/ml during infection. To obtain values within range of the standard curve we suggest that samples be diluted 2500-fold using the following procedure for each sample to be tested:

1. Dispense 245 μl of diluent into two microcentrifuge tubes.
2. Pipette and mix 5.0 μl of the serum/plasma sample with 245 μl of diluent in the first tube This provides a 50-fold dilution.
3. Pipette and mix 5.0 μl of the 50-fold diluted sample with 245 μl of diluent in the second tube This provides a 2500-fold dilution.

CONJUGATE PREPARATION

Shortly before it is required (step 6 in the procedure section), prepare the conjugate as described on the stock vial label, by mixing the indicated volumes of stock and diluent.

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 4°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 freshly prepared 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.

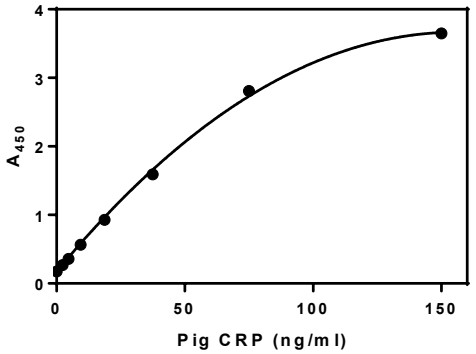
CALCULATION OF RESULTS

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 total and nonspecific two site binding model) 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 the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

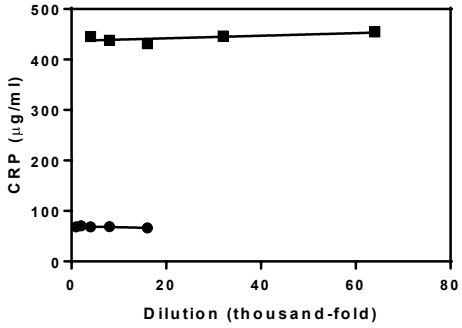
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CRP (ng/ml)	Absorbance (450 nm)
150	3.647
75	2.809
37.5	1.593
18.75	0.929
9.38	0.565
4.67	0.358
2.34	0.269
0	0.174



LINEARITY

To assess linearity of the assay, serum samples with CRP levels of 69 and 443 µg/ml were serially diluted to produce values within range of the assay.



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3. Pomorska-Mol M, Kwik K and Markowska-Daniel I. Major acute phase proteins in pig serum from birth to slaughter. Bull Vet Inst Pulawy 56:553-557 (2012)
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***PROTOCOL AND MATERIALS MODIFIED 12/23/2020**

For technical assistance please email us at techsupport@lifediagnostics.com