KAMIYA BIOMEDICAL COMPANY

Pig High-Sensitive CRP ELISA

For the quantitative determination of C-reactive protein in pig serum or plasma

Cat. No. KT-184

For Research Use Only.

PRODUCT INFORMATION

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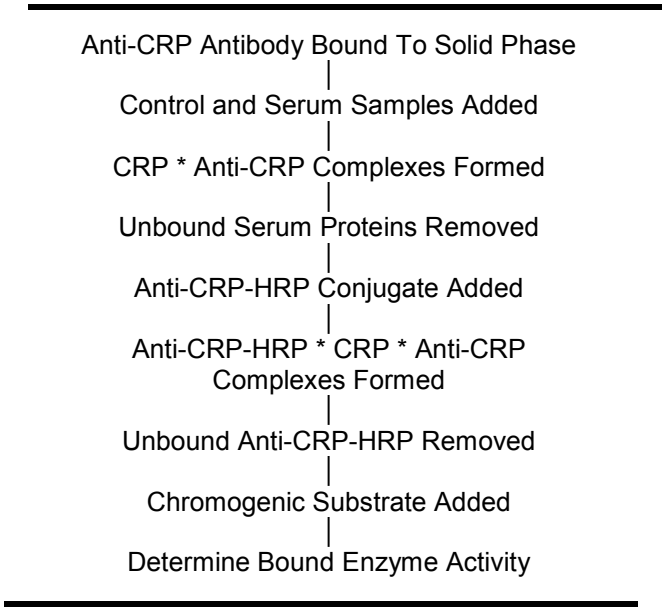
INTENDED USE
The Pig High-Sensitive C-Reactive Protein ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring C-Reactive Protein (CRP) in serum or plasma of pigs. For research use only.

INTRODUCTION
Acute phase proteins are plasma proteins which increase in concentration following infection, inflammation or trauma. The first acute phase protein to be recognized was discovered in humans by Tillet and Frances in 1930. This CRP is so named because it is able to effect precipitation of somatic C-polysaccharide of *Streptococcus pneumonia.* CRP is an alpha globulin with a mass of 110,000 to 140,000 daltons, and composed of five identical subunits, which are non-covalently assembled as a cyclic pentamer. It is synthesized in the liver and, in humans, is normally present as a trace constituent of serum at a level less than 0.3 mg/dL. The CRP levels in serum rise quickly following acute tissue damage within 24 to 48 hours. It also falls very rapidly once the stimulus is removed. It has been proposed that CRP aids in complement activation, influences phagocytic cell function, and augments cell-mediated cytotoxicity. Investigations over the past few years have shown that quantification of CRP in plasma or serum can provide valuable information in the detection, prognosis, and monitoring of disease not only in humans, but in companion animals and farm herds as well.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the CRP present in the serum sample reacts with the anti-CRP antibody, which has been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound serum proteins by washing, anti-CRP antibody conjugated with horseradish peroxidase (HRP) is added. This HRP-conjugated antibody forms a complex with the previously bound serum CRP. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to the concentration of CRP in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of CRP in the test sample. The quantity of CRP in the test sample can be interpolated from the calibration curve constructed from the calibrators and corrected for serum dilution.

Figure 1.



COMPONENTS

1. Diluent
   One bottle containing 50 mL of a 5X concentrated phosphate buffered saline (PBS) solution with 0.25% Tween and 0.1% Proclin 300 as a preservative.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 10X concentrated PBS solution with 0.5% Tween.

3. Enzyme-Antibody Conjugate
   One vial containing 200 μL of affinity purified anti-pig CRP antibody conjugated with HRP in stabilizing buffer.

4. Substrate Solution
   One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

5. Stop Solution
   One vial containing 12 mL 0.3 M sulfuric acid. WARNING: Avoid contact with skin.

6. Microtiter Plate
   12 removable eight (8)-well strips in well holder frame. Wells are coated with affinity purified anti-pig CRP.

7. Pig CRP Calibrator
   One vial containing a lyophilized Pig CRP Calibrator

8. Positive Control
   One vial containing 50 μL of serum with 0.1% sodium azide. See the control certificate for the concentration and suggested dilution.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 μL to 200 μL)
- Test tubes
- Microplate washer/aspirator
- Distilled or deionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives
   Diluent contains 0.1% Proclin 300 as a preservative. Proclin 300 is not toxic at the concentration used in the kit.
5. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
6. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
7. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent
   The Diluent supplied is a 5X concentrate and must be diluted 1:5 with distilled or deionized water. Mix gently before use. Avoid foaming.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 10X concentrate and must be diluted 1:10 with distilled or deionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate
   The required amount of working conjugate solution for each microtiter plate is prepared by adding 100 µL Enzyme-Antibody Conjugate to 10 mL of Diluent. Mix uniformly, but gently. Avoid foaming.

4. Substrate Solution
   Ready to use as supplied.

5. Stop Solution
   Ready to use as supplied.

6. Microtiter Plate
   Ready to use as supplied.

7. Pig CRP Calibrators
   Add 1.3 mL of distilled or de-ionized water to the Pig CRP Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 2.1 µg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Pig CRP calibrators need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

Standard	ng/mL	Amount →	1 x Diluent
1	100	40 µL Pig CRP Calibrator	800 µL
2	50	0.3 mL Calibrator 1	0.3 mL
3	25	0.3 mL Calibrator 2	0.3 mL
4	12.5	0.3 mL Calibrator 3	0.3 mL
5	6.25	0.3 mL Calibrator 4	0.3 mL

8. Positive Control
   The concentration and recommended dilution are provided on the control certificate.

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: if you are storing the kit for more than 7 days, store the CRP Calibrator and Control frozen at -20°C.**

2. Diluent
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution
   The 10X Wash Solution Concentrate should be stored at 4°C and is stable until the expiration date. The working solution should be stored at room temperature (RT, 16-25°C) or 4°C and is stable for at least one week from the date of preparation.

4. Enzyme-Antibody Conjugate
   Undiluted anti-CRP-HRP conjugate should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for one day at 4°C.

5. Substrate Solution
   The Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate
   Anti-Pig CRP coated wells are stable until the expiration date, and should be stored at 4°C in the sealed foil pouch with desiccant pack.

8. Pig CRP Calibrator
   For storage **longer than 7 days, keep frozen** until the expiration date. If storing for less than 7 days, the calibrator can be stored at 4°C. The reconstituted calibrator should be aliquoted and stored frozen. Aliquot to avoid multiple freeze/thaw cycles. The working standard solutions should be prepared immediately prior to use and are stable for 1 day.

9. Positive Control
   One vial containing 50 μL of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Specimens may be shipped at RT and then stored refrigerated at 4°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.
No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Serum Samples

Due to the high sensitive nature of the assay each serum sample should be diluted before use for a normal assay. For a single step determination a dilution of serum at 1:2,000 is appropriate for most samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required.

To prepare a 1:2,000 serum dilution, transfer 5 μL of sample to 495 μL of diluent. This gives you a 1:100 dilution. Mix thoroughly. Next, dilute the 1:100 samples by transferring 20 μL to 380 μL of diluent. You now have a 1:2,000 dilution of your sample. Mix thoroughly at each stage.

Procedure

Bring all reagents to RT before use.

1. Add 100 μL of Diluent to each of the wells in A1 & A2. These will serve for an evaluation of the background associated with the assay.

2. Pipette 100 μL of
   Calibrator 1 (100 ng/mL) into wells B1 & B2
   Calibrator 2 (50 ng/mL) into wells C1 & C2
   Calibrator 3 (25 ng/mL) into wells D1 & D2
   Calibrator 4 (12.5 ng/mL) into wells E1 & E1
   Calibrator 5 (6.15 ng/mL) into wells F1 & F1
3. Pipette 100 μL of diluted Positive Control into wells G1 & G2.

4. Pipette 100 µL of serum sample (test sample 1) into wells H1 & H2. The next sample goes in wells A3 & A4, the next in B3 & B4 and so on.

5. Incubate the microtiter plate at 22°C (RT) for thirty (30 ± 2) minutes. Keep plate level during incubation.

6. Following incubation, aspirate the contents of the wells.

7. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer.

8. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate in the dark at 22°C (RT) for thirty (30 ± 2) minutes.

9. Wash and blot the wells as described in Steps 6 and 7.

10. Pipette 100 µL of TMB Substrate Solution into each well.

11. Incubate in the dark at RT for precisely ten (10) minutes.

12. After ten minutes, add 100 µL of Stop Solution to each well.

13. Determine the absorbance at 450 nm. Zero the plate reader to air. The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a second order polynomial (quadratic).

3. Interpolate test sample values from calibration curve. Correct for serum dilution factor to arrive at CRP concentration in original sample.

EXPECTED VALUES

The CRP concentration in normal pig serum has not yet been firmly established.

KNOWN INTERFERING SUBSTANCES

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

PERFORMANCE CHARACTERISTICS

In accord with good laboratory practice, the assays for specific CRP require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of redistilled or deionized water, and accuracy of reagent and sample pipetting.

FOR RESEARCH USE ONLY

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