# PIG C-Reactive Protein (CRP) Assay Kit

Catalog Number: PPE51-K01

For Research Use Only. Not for Diagnostic Purposes

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## INTENDED USE

The CRP test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring CRP in pig biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

*For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.*

## ASSAY BACKGROUND

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 Human by Tillet and Frances in 1930. This C-reactive protein (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 Human, is normally present as a trace constituent of serum at levels less that 0.3 mG/dL. The levels in serum rise quickly following acute tissue damage and also falls very rapidly once the stimulus is removed. It has been proposed that the function of CRP is to aid in complement activation, influence phagocytic cell function, and augment cell mediated cytotoxicity. Investigations over the past few years have shown that quantification of these in plasma or serum can provide valuable diagnostic information in the detection, prognosis, and monitoring of disease not only in Human, but in companion animals and farm herds as well.

## ASSAY PRINCIPLES

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the c-reactive protein (CRP) present in samples reacts with the anti-CRP antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-CRP antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound 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 varies directly with 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 standard curve constructed from the standards, and corrected for sample dilution.

## REAGENTS PROVIDED AND REAGENT PREPARATION

Store all other reagents at 2 to 8°C. Use only reagents supplied with this Salivary Cortisol ELISA Assay kit. Do not interchange reagents with different lot numbers. Expiration dates and lot numbers are printed on the labels.

| Component | Description | Preparation | Storage | Stability |
|---|---|---|---|---|
| **ELISA Micro Plate, antibody coated** | One plate of 12 removable 8 well strips, antibody coated | Ready to use as supplied. | 2-8°C, In sealed foil bag with desiccant | With proper storage the plate strips are stable until the expiration date. |
| **Enzyme Conjugated Detection Antibody** | One vial of 150µL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer | Dilute 1/100 immediately prior to use. | 2-8°C in the dark | The working conjugate solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date. |
| **Calibrator** | One vial of calibrator | Refer to the Certificate of Analysis (CoA). | 2-8°C for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles. | The working standard solutions should be prepared immediately prior to use. |
| **Diluent Solution** | One 60 mL bottle of 1X diluent buffer | Ready to use as supplied | 2-8°C | The 1X solution is stable until the expiration date. |
| **Wash Solution Concentrate** | One 50 mL bottle of 20X wash solution | Dilute 1/20 to make 1X working solution. | 2-8°C for both 1X working solution and 20X concentrate | The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date. |
| **Chromogen-Substrate Solution** | One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3. | Ready to use as supplied | 2-8°C in the dark | Protect from light. The Substrate Solution is stable until the expiration date. |
| **STOP Solution WARNING: Avoid Contact with Skin** | One 12 mL bottle of 0.3 M sulfuric acid. | Ready to use as supplied | 2-8°C | The Stop Solution is stable until the expiration date. |

## STORAGE AND STABILITY

- When stored at 2-8C, unopened reagents will retain activity until the expiration date. Do not use reagents beyond this date
- Use only reagents supplied with this kit. Do not interchange reagents with different lot numbers
- Opened reagents must be stored at 2-8C
- Microtiter wells must be stored at 2-8C. Once foil bag has been opened, care should be taken to reseal tightly.
- Opened kits retain activity one (1) month if stored properly
- Expiration dates and lot numbers are printed on the labels

## MATERIALS NEEDED BUT NOT SUPPLIED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate Reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection

- Timer

## SPECIMEN COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.
If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.
The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- **Serum samples** - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at –80C (preferably) or -20C. Avoid repeated freeze-thaw cycles.

- **Plasma samples** - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at –80C (preferably) or -20C. Avoid repeated freeze-thaw cycles.

- **Urine samples** – Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at –80C (preferably) or -20C. Avoid repeated freeze-thaw cycles.

- **Known interfering substances** - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction. EDTA may affect CRP binding; therefore, is not recommended for use as an anti-coagulant.

## DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- **Serum samples** – Recommended starting dilution is 1/2,000. To prepare a 1/2,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 20 µL into 380 µL of 1X diluent. This gives you a 1/2,000 dilution. Mix thoroughly each stage.

- **Plasma samples** – Recommended starting dilution is 1/2,000. To prepare a 1/2,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 20 µL into 380 µL of 1X diluent. This gives you a 1/2,000 dilution. Mix thoroughly each stage

## REAGENT PREPARATION

- Bring all reagents to room temperature (16C to 25C) before use.

- Diluent Solution - Ready to use as supplied.

- Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35C before dilution can dissolve crystals.

- Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

- Pig CRP Calibrator – Prepare according to the lot specific Certificate of Analysis.

**ASSAY PROCEDURE**

1. **All samples and standards should be assayed in duplicates.**

2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

**Pipette 100 µL of**

Standard 0 (0.0 ng/mL) in duplicate
Standard 1 (6.25 ng/mL) in duplicate
Standard 2 (12.50 ng/mL) in duplicate
Standard 3 (25 ng/mL) in duplicate
Standard 4 (50 ng/mL) in duplicate
Standard 5 (100 ng/mL) in duplicate
Standard 6 (200 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre-designated wells.

4. Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

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

6. 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 wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in steps 5 / 6.

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

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

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

12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate reader to manufacturer's specifications.

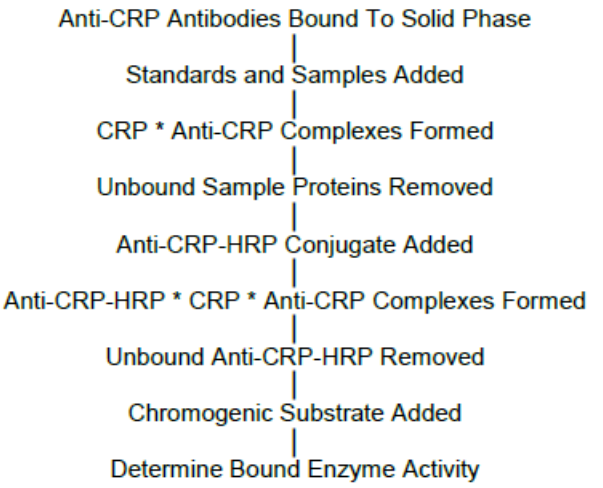
## CALCULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.

2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the CRP concentration in original samples.

**Quick Assay Principle**



## WARRANTY INFORMATION

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*For further information about this kit, its application, or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*