

KAMIYA BIOMEDICAL COMPANY

Rabbit High-Sensitive CRP ELISA

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

Cat. No. KT-097

For research use only.

PRODUCT INFORMATION**Rabbit High-Sensitive CRP ELISA**
Cat. No. KT-097**INTENDED USE**

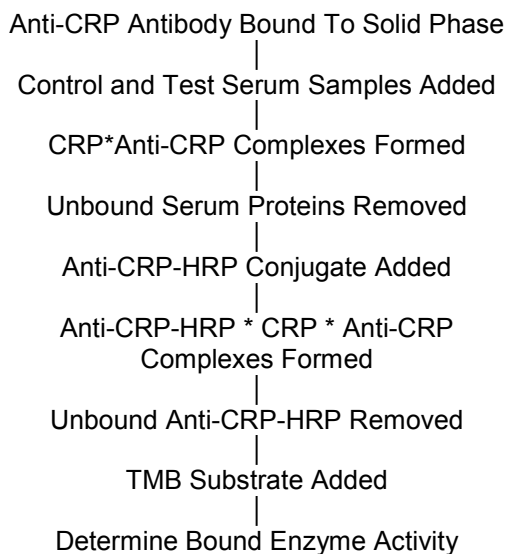
The Rabbit High-Sensitive CRP ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring C-reactive protein (CRP) in serum or plasma of rabbits. For research use only.

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 pneumoniae*. 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 level in serum rises quickly following acute tissue damage and can reach a level 1,000-fold higher 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 phosphate buffered saline (PBS) solution with 0.05% Tween, protein stabilizer 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 μ L of a 100X concentrated affinity purified chicken anti-rabbit CRP antibody conjugated with HRP in a stabilizing buffer.
4. TMB 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 of 0.3 M sulfuric acid. WARNING: Avoid contact with skin.
6. Microtiter Plate
Twelve removable eight (8)-well micro strips in well holder frame. Wells are coated with affinity-purified chicken anti-rabbit CRP.
7. Rabbit CRP Calibrators
Four vials (1.2 mL/vial) containing pre-diluted calibrator solutions. Calibrator 1 is adjusted to contain 50 ng/mL of rabbit CRP; Calibrator 2, 3 and 4 are serial two-fold dilutions of Calibrator 1.
8. Positive Control
One vial containing 50 μ L of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 μ L to 200 μ L)
- Test tubes
- Microplate washer/aspirator
- Distilled or deionized H₂O
- 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
Ready to use. 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. TMB Substrate Solution
Ready to use as supplied.
5. Stop Solution
Ready to use as supplied.
6. Microtiter Plate
Ready to use as supplied.
7. Rabbit CRP Calibrators
Ready to use. Mix gently before use. Avoid foaming. For samples containing very low levels of CRP, it is possible to extend the utility of the lower detection limit of this assay by further serial 2-fold dilution of Calibrator 4.
8. Positive Control
The concentration and recommended dilution provided are on the Control Certificate.

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. Diluent
The Diluent should be stored at 4°C and is stable until the expiration date.
2. Wash Solution Concentrate
The 10X Wash Solution Concentrate is stable at 4°C until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4°C.
3. Enzyme-Antibody Conjugate
Undiluted horseradish peroxidase anti-CRP conjugate should be stored at 4°C and dilute immediately prior to use. The working conjugate solution is stable for one day.
4. TMB Substrate Solution
The Substrate Solution should be stored at 4°C and is stable until the expiration date.
5. Stop Solution
The Stop Solution should be stored at 4°C and is stable until the expiration date.
6. Microtiter Plate
Anti-rabbit CRP coated wells are stable until the expiration date, and should be stored at 4°C in the sealed foil pouch with desiccant pack.
7. Rabbit CRP Calibrators
For storage longer than 7 days keep frozen until the expiration date. Storage of less than 7 days can be at 4°C. Avoid multiple freeze-thaw cycles.
8. Positive Control
For storage longer than 7 days keep frozen until the expiration date. Storage less than 7 days can be at 4°C. Avoid multiple freeze/thaw cycles.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values shown in figure 2.

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 room temperature (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 1:400 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 1:400 serum dilution, first transfer 5 µL of sample to 195 µL of diluent (1:40). Mix thoroughly. Next take 40 µL of the 1:40 dilution and add 360 µL of diluent. Make sure to mix thoroughly at each stage. These steps will yield a 1:400 dilution of your sample.

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 into wells B1 & B2
 - Calibrator 2 into wells C1 & C2
 - Calibrator 3 into wells D1 & D2
 - Calibrator 4 into wells E1 & E2
3. Pipette 100 µL of diluted Positive Control into wells F1 & F2.
4. Pipette 100 µL of diluted serum sample (test sample 1) into wells G1 & G2. The next sample goes in wells H1 & H2, the next in A3 & A4 and so on.
5. Incubate the Microtiter Plate at 22°C (RT) for ten (10 ± 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 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. Repeat three times for a total of four washes.
8. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) in the dark for ten (10 ± 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 five (5) minutes.

12. After five minutes, add 100 μ L of Stop Solution to each well.
13. Determine the absorbance at 450 nm of the contents of each well. 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

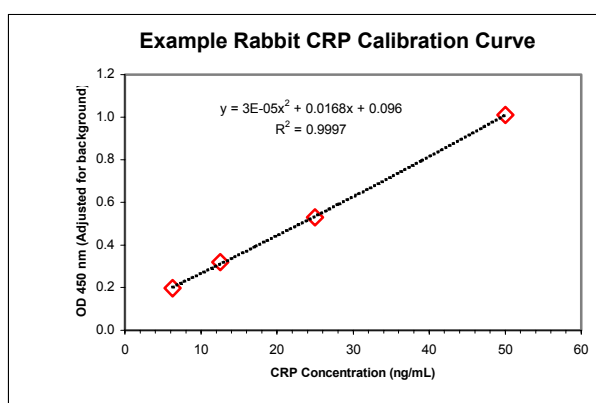
1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
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 rabbit serum has not yet been firmly established.

Figure 2. Typical Calibration Curve for Rabbit High-Sensitive CRP ELISA

(This curve is only an example. Each user must make his own calibration curve.)



KNOWN INTERFERING SUBSTANCES

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

QUALITY CONTROL

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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