

HIGH SENSITIVITY C-REACTIVE PROTEIN ENZYME IMMUNOASSAY TEST KIT

Catalog Number: BC-1119



BioCheck, Inc
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High Sensitivity Enzyme Immunoassay for the Quantitative Determination of C-Reactive Protein Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

BioCheck hsC-Reactive Protein Enzyme Immunoassay (hsCRP ELISA)

INTENDED USE

The hsCRP ELISA is intended for the quantitative determination of C-reactive protein (CRP) in human serum. Enhanced sensitivity measurements of CRP can be useful for the detection and evaluation of infection, tissue injury, inflammatory disorders and associated diseases.

SUMMARY AND EXPLANATION OF TEST

C-Reactive protein (CRP) was identified by Tilet and Francis (1930) in the plasma of patients with pneumonia, and was named for its ability to bind and precipitate the C-polysaccharide of pneumococcus.^{1,2} It is an alpha globulin with a molecular mass of approximately 110,000 to 140,000 daltons, and is composed of five identical subunits, which are noncovalently assembled as a cyclic pentamer.³ CRP is synthesized in the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl.^{2,4,5,6} Its physiological roles are numerous and varied, but with several functions similar to those of immunoglobulins, CRP appears to function in host defense.¹

CRP is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases. This include infections by gram-positive and gram-negative organisms, acute phase of rheumatoid arthritis, abdominal abscesses, and inflammation of the bile duct.³ CRP may also be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases, burned patients and after surgical trauma.^{3,7,8}

Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes.⁹ CRP levels rise in serum or plasma within 24 to 48 hours following acute tissue damage, reach a peak during the acute stage (approximately 1000x constitutive level) and decrease with the resolution of inflammation or trauma.^{1,10,11} The concentration increase of CRP in human serum or plasma may last for several days before decreasing to normal levels.¹⁰⁻¹²

The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate¹³, which may also be influenced by physiological changes not associated with an inflammatory process. Current testing methods including latex agglutination, nephelometry, and radial immunodiffusion (RID) have the general disadvantages of low sensitivity, whereas enzyme-linked immunosorbent assays (ELISA) provide the highest sensitivity and specificity.¹⁴⁻¹⁶

As elevated CRP values are always associated with pathological changes, the CRP assay provides useful information for the diagnosis, therapy and monitoring of inflammatory processes and associated disease.¹⁰⁻¹³ Additionally, measurement of CRP by high-sensitivity CRP assays may add to the predictive value of other cardiac markers (myoglobin, creatine-kinase-MB, troponin I and T), which are used to assess the risk of cardiovascular and peripheral vascular disease.¹⁷⁻²³ As increases in CRP values are non-specific, they should not be interpreted without a complete patient history evaluation, and measurements of CRP should be compared to previous values.

PRINCIPLE OF THE ASSAY

The hsCRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay.²⁴ The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of CRP is directly proportional the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS AND MATERIALS PROVIDED

1. Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with mouse monoclonal anti-CRP.
2. Reference Standard Set (1.0 ml/vial)
Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/l CRP in phosphate buffer-BSA solution with preservatives; lyophilized.
3. hsCRP Sample Diluent (50 ml/vial)
Contains phosphate buffer-BSA solution with preservatives.
4. CRP Enzyme Conjugate Reagent (12 ml/vial)
Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.
5. TMB Reagent (11 ml/bottle)
Contains one-step TMB solution.
6. Stop Solution (1 bottle, 11 ml/bottle)
Contains diluted hydrochloric acid (1N HCl).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.²⁵
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. ***Patient serum should be diluted 100 fold prior to use. Prepare a series of small tubes (i.e., 1.5 ml microcentrifuge tubes) and mix 5 µl of serum with 495 µl (0.495 ml) Sample Diluent. DO NOT DILUTE THE STANDARDS.***
3. Samples with expected CRP concentrations over 10 mg/l may be quantitated by further dilution (10 fold) of the 100-fold diluted solution with sample diluent (i.e., 10 µl of the 100-fold diluted sample to 90 µl sample diluent).
4. Reconstitute each lyophilized standard with 1.0 mL dH₂O. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for up to 30 days.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells *within 60 minutes* after collection.
3. Specimens which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. ***Patient serum and control serum should be diluted 100 fold prior to use. See Reagent Preparation. PLEASE DO NOT DILUTE THE STANDARDS.***
2. Secure the desired number of coated wells in the holder.
3. Dispense 10 µl of CRP standards, DILUTED specimens, and DILUTED controls into appropriate wells.
4. Dispense 100 µl of CRP Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25 °C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. DO NOT USE TAP WATER.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

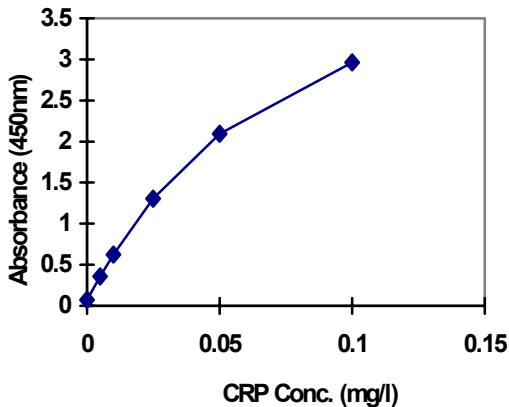
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD₄₅₀) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mg/l on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP (mg/l) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
5. Patient samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/l.
6. NOTE: Patient samples with CRP concentrations less than 0.1 mg/l should reported as "<0.1 mg/l CRP".

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against CRP concentrations shown on the X axis. **NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

| CRP (mg/l) | Absorbance (450 nm) |
|------------|---------------------|
| 0 | 0.073 |
| 0.005 | 0.358 |
| 0.010 | 0.624 |
| 0.025 | 1.305 |
| 0.050 | 2.093 |
| 0.100 | 2.962 |



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding

of the package insert instructions and with adherence to good laboratory practice.

2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Patient samples may contain human anti-mouse antibodies (HAMA) that are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal range based on the patient population. However, based on published literature healthy individuals are expected to have CRP values as follows:²⁶

Neonatal serum: 0.01 to 0.35 mg/l
Adult serum: 0.068 to 8.2 mg/l

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using 117 human serum samples, ranging in CRP concentration from 0.62 mg/l to 119.3 mg/l, demonstrated good correlation with a commercially available kit as shown below.

Comparison between the hsCRP ELISA and the Dade-Behring N *High Sensitivity* CRP test provided the following data (n=117):

Correlation coefficient = 0.9594
 Slope = 0.8396
 Intercept = 1.3948
 Mean = 13.74 mg/l
 Dade Mean = 14.75 mg/l

PERFORMANCE CHARACTERISTICS

2. Sensitivity

The minimum detectable concentration of the CRP ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.1 mg/l. Additionally, the functional sensitivity was determined to be 0.1 mg/l ((as determined with inter-assay %C.V. < 20%). **Lower limit of hsCRP ELISA \cong 0.1 mg/l CRP; upper limit = 10 mg/l CRP.**

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of five different serum samples in one assay. Within-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 | 4 | 5 |
|-----------------|-------|-------|-------|-------|--------|
| # Replicates | 22 | 22 | 22 | 22 | 20 |
| Mean CRP (mg/l) | 0.546 | 0.894 | 2.021 | 3.492 | 17.549 |
| S.D. | 0.041 | 0.037 | 0.085 | 0.146 | 0.397 |
| C.V. (%) | 7.5% | 4.1% | 4.2% | 4.1% | 2.3% |

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

| Serum Sample | 1 | 2 | 3 | 4 | 5 |
|-----------------|-------|-------|-------|-------|--------|
| # Replicates | 20 | 20 | 20 | 20 | 20 |
| Mean CRP (mg/l) | 0.490 | 0.890 | 1.925 | 3.529 | 17.435 |
| S.D. | 0.020 | 0.023 | 0.078 | 0.114 | 0.438 |
| C.V. (%) | 4.1% | 2.5% | 4.1% | 3.2% | 2.5% |

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known CRP levels were combined and assayed in duplicate. The mean recovery was 100.4%.

| PAIR NUMBER | EXPECTED [CRP] (mg/l) | OBSERVED [CRP] (mg/l) | % RECOVERY |
|-------------|-----------------------|-----------------------|------------|
| 1 | 0.600 | 0.606 | 101% |
| 2 | 1.218 | 1.269 | 104% |
| 3 | 2.724 | 2.528 | 93% |
| 4 | 3.635 | 3.408 | 94% |
| 5 | 4.633 | 4.787 | 103% |
| 6 | 5.740 | 6.319 | 110% |
| 7 | 8.721 | 8.587 | 98% |

b. Linearity

Five patient samples were serially diluted to determine linearity. The mean recovery was 99.4%.

| # | Dilution | Expected Conc. (mg/l) | Observed Conc. (mg/l) | % Expected | |
|---------------|--------------|-----------------------|-----------------------|------------|------|
| 1. | Undiluted | ---- | ---- | ---- | |
| | 1:2 | ---- | ---- | ---- | |
| | 1:4 | 5.150 | 4.900 | 95.1% | |
| | 1:8 | 2.575 | 2.632 | 102.2% | |
| | 1:16 | 1.288 | 1.303 | 101.1% | |
| | 1:32 | 0.644 | 0.594 | 92.2% | |
| | 1:64 | 0.320 | 0.340 | 106.3% | |
| | 1:128 | 0.160 | 0.170 | 106.2% | |
| | 1:256 | 0.080 | 0.073 | 91.3% | |
| | Mean = 99.2% | | | | |
| 2. | Undiluted | ---- | ---- | ---- | |
| | 1:2 | ---- | ---- | ---- | |
| | 1:4 | ---- | ---- | ---- | |
| | 1:8 | 6.691 | 6.625 | 93.4% | |
| | 1:16 | 3.346 | 3.338 | 99.8% | |
| | 1:32 | 1.672 | 1.600 | 95.7% | |
| | 1:64 | 0.836 | 0.818 | 97.8% | |
| | 1:128 | 0.418 | 0.441 | 105.5% | |
| | 1:256 | 0.209 | 0.224 | 107.2% | |
| | 1:512 | 0.105 | 0.109 | 103.8% | |
| Mean = 100.5% | | | | | |
| 3. | Undiluted | ---- | ---- | ---- | |
| | 1:2 | ---- | ---- | ---- | |
| | 1:4 | 6.484 | 6.578 | 101.4% | |
| | 1:8 | 3.242 | 3.325 | 102.5% | |
| | 1:16 | 1.621 | 1.613 | 99.5% | |
| | 1:32 | 0.811 | 0.788 | 97.0% | |
| | 1:64 | 0.405 | 0.372 | 91.9% | |
| | 1:128 | 0.203 | 0.210 | 103.4% | |
| | Mean = 99.3% | | | | |
| | 4. | Undiluted | ---- | ---- | ---- |
| 1:2 | | ---- | ---- | ---- | |
| 1:4 | | ---- | ---- | ---- | |
| 1:8 | | 6.080 | 6.498 | 106.9% | |
| 1:16 | | 3.040 | 3.336 | 109.7% | |
| 1:32 | | 1.520 | 1.610 | 105.9% | |
| 1:64 | | 0.760 | 0.761 | 100.1% | |
| 1:128 | | 0.380 | 0.345 | 90.8% | |
| 1:256 | | 0.190 | 0.176 | 92.6% | |
| 1:512 | | 0.094 | 0.090 | 95.7% | |
| Mean = 100.2% | | | | | |
| 5. | Undiluted | ---- | ---- | ---- | |
| | 1:2 | ---- | ---- | ---- | |
| | 1:4 | 6.055 | 5.995 | 99.0% | |
| | 1:8 | 3.028 | 2.877 | 95.0% | |
| | 1:16 | 1.514 | 1.413 | 93.3% | |
| | 1:32 | 0.757 | 0.695 | 91.8% | |
| | 1:64 | 0.378 | 0.394 | 104.2% | |
| | 1:128 | 0.189 | 0.178 | 94.2% | |
| | 1:256 | 0.094 | 0.102 | 108.5% | |
| | 1:512 | 0.047 | 0.045 | 95.7% | |
| Mean = 97.7% | | | | | |

PERFORMANCE CHARACTERISTICS

5. Specificity

The following analytes were tested for cross-reactivity:

| MATERIAL TESTED | TEST CONCENTRATION |
|---------------------|---------------------------------|
| Bilirubin | 50 mg/l 100 mg/l 230 mg/l |
| Hemoglobin | 12 g/l 24 g/l 36 g/l |
| Triglyceride | 2.5 g/l 5.0 g/l 7.5 g/l |
| Human IgG | 5 g/l 10 g/l 25 g/l |

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