CRP (Human) ELISA Kit

Catalog Number KA0238

96 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

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.

Background

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 rate[13], 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 CRP (Human) 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 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.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-Coated Wells: Microtiter wells coated with mouse monoclonal anti-CRP.	96 wells
Reference Standard Set: Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/L CRP in serum based buffer with preservatives.	1 mL x 6
hsCRP Sample Diluent: Contains phosphate buffer-BSA solution with preservatives.	50 mL
CRP Enzyme Conjugate Reagent: Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.	12 mL
TMB Reagent (One-Step)	11 mL
Stop Solution (1N HCl)	11 mL

Storage Instruction

- ✓ 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.
- ✓ Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Materials Required but Not Supplied

- ✓ Precision pipettes: 5 µL, 10 µL, 50 µL, 100 µL and 1.0 mL
- ✓ Disposable pipette tips
- ✓ Microtiter well reader capable of reading absorbance at 450 nm.
- ✓ Vortex mixer, or equivalent
- ✓ Absorbent paper
- ✓ Graph paper

Precautions for Use

- Warnings and Precaution
- ✓ 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]

- ✓ 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.
- ✓ Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
- ✓ Replace caps on reagents immediately. Do not switch caps.
- ✓ Do not pipette reagents by mouth.

- Limitation of procedures
- ✓ 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.
- ✓ The results obtained from the use of this kit should be used for research only, not for diagnostic procedures.
- ✓ Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- ✓ The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- ✓ 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 human specimens cannot be guaranteed.

Assay Protocol

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum sample 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).

Sample 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.

Assay Procedure

1. Serum sample 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.

Note:

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

- **Quality control**

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

Data Analysis

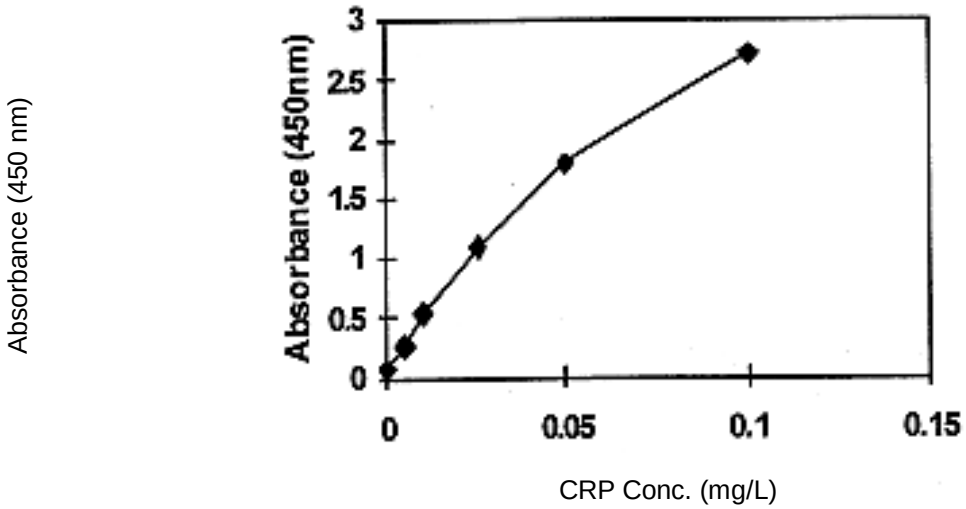
Calculation of Results

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 samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/L.
5. 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.
   NOTE: 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 450 nm 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	0.066
0.005	0.264
0.010	0.457
0.025	1.092
0.050	1.788
0.100	2.710



Performance Characteristics

- Expected values

It is recommended that each laboratory establish its own normal range based on the human 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

- 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 CRP (Human) 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

- 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 CRP (Human) ELISA ≅ 0.1 mg/L CRP; upper limit = 10 mg/L CRP.

- Precision

✓ 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%

✓ 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%

✓ **Recovery**

Various 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%

- Linearity

Five human 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%

- 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

Resources

References

1. Schultz, D.R., and Arnold P.I.: “Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, glycoprotein, and fibrinogen.” Seminars in Arthritis and Rheumatism 20: 129-147, 1990.
2. Kindmark, C.O.: The Concentration of C-reactive protein in Sera from Healthy Individuals. Scand J Clin Lab Invest, 29: 407- 411, 1972.
3. Dowling, P., and Cook, S.: Immune events in demyelinating disease. In Wolfgang, F., Ellison, G.W., Stevens J.G., and Andrew, J.M. (eds.): Multiple sclerosis. Academic Press Inc., New York, 269-277, 1972.
4. Yudkin, J.S., et. al.: C-Reactive Protein in Healthy Subjects: Association with Obesity, Insulin Resistance, and endothelial dysfunction. A potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 19:972-8, 1999.
5. Kushner, I., Rzewnicki, D.L.: The acute phase response: General aspects. Bailliere’s Clinical Rheumatology 8: 513-530, 1994.
6. Macy, E.M., Hayes, T.E., and Tracy, R.P.: Variability in the measurement of C-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin Chem, 43;1:52-58, 1997.
7. Hedlund, P.: Clinical and experimental studies on C-reactive protein (acute phase protein). Thesis Acta Med Scand, 128 (Suppl, 361):1-71, 1961.
8. Hedlund, P.: The appearance of acute phase protein in various diseases. Acta Med Scand, 128, (Suppl,196): 579-601, 1947.
9. Morley, J.J., Kushner, I.: Serum C-reactive Protein Levels. in: Kushner, I., Volanakis, J.E., and Gerwutz, H., eds., C-Reactive Protein and the Plasma Protein Response to Tissue Injury. Annals of N.Y. Acad Sci, 389: 406-417, 1982.
10. Shine, B., de Beer, F.C., and Pepys, M.B.: Solid phase radiommunoassays for human C-reactive protein. J Lab Clinica Chimica Acta 117:13-23, 1981.
11. Kushner, I.: “C-reactive protein in rheumatology,” Arthritis Rheum 34:1065-1068, 1991.
12. Dixon, J.S., et al.: C-reactive protein in the serial assessment of disease activity in rheumatoid arthritis. Scand J Rheum, 13: 39-44, 1984.
13. Hind, C.R.H., and Pepys, M.B.: The role of serum C-reactive (CRP) measurement in clinical practice. Int Med. 5: 112-151, 1984.
14. Van Leeuwen, M., and Van Rijswijk, M.H.: Acute phase proteins in monitoring inflammatory disorders. Bailliere’s Clinical Rheumatology 8: 531-552, 1994.
15. Powell, L., Roantree, R.J., and Rantz, L.A.: C-reactive Protein. A review. Clinical experience with the C-reactive protein test. Am J Med Technol, 87: 138-142, 1979.
16. Roberts, W. L., et. al.: Evaluation of Four Automated High-Sensitivity C-Reactive Protein Methods:

Implications for Clinical and Epidemiological Applications. Clin Chem 46:4, 461-468, 2000.

17. Ridker, P.M., et. al.: Plasma concentration of C-reactive protein and the risk of developing peripheral vascular disease. Circulation, 97:425-428, 1998.
18. Ridker, P.M., et. al.: Propestive study of C-reactive protein and the risks of future cardiovascular events among apparently healthy women. Circulation, 98:731-733, 1998.
19. Ridker, P.M., Glynn, R.J., and Hennekens, C.H.: C-reactive protein adds to the predicitive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation, 97:2007-2011, 1998.
20. Ridker, P.M., et. al.: Inflammation, Pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Circulation, 98:839-844, 1998.
21. Tracy, R.P., et. al.: Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results fro the Cardiovascular Health Study and the Rural Health Promotion Project.. Arter. Thromb. Vasc. Biol. 17:1121-1127, 1997.
22. Macy, E. M., Hayes, T.E., Tracy, R.P.: Variability in the measurement of C-reactive protein in healthy subjects: implications for reference interval and epidemiological applications. Clin. Chem. 43(1):52-58, 1997.
23. Ridker, P.M., et. al.: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N. Engl. J. Med., 336:973-979, 1997.
24. Votila, M., et. el.: Immunol Methods, 42: 11, 1981.
25. USA Center for Disease Control/National Institute of Health Manual, “Biosafety in Microbiological and Biomedical Laboratories”, (1984).
26. “Clinical Guide to Laboratory Tests”. Edited by N.W. Tietz, 3rd Edition. W.B. Saunders Company, Philadelphia, PA. 19106 (1995).

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H