9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

C-Reactive Protein ELISA Kit

Name	C-Reactive Protein ELISA Kit
INTENDED USE	The C-Reactive Protein Ultra Sensitive ELISA Kit is intended for the quantitative determination of C-reactive protein (CRP) in human serum or plasma.
SUMMARY AND EXPLANATION	C-Reactive protein (CRP) 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. 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. 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. 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. 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. Inflammation in the arteries may play a role in heart disease and HS-CRP can determine heart disease risk in those with undetected heart disease and risk of complications for those who have already had a heart event Principle of the Assay The CRP ELISA kit is a solid phase direct sandwich method. The diluted samples and conjugate reagent (anti-CRP Biotin & HRP) are added to the wells coated with Streptavidin. CRP in the patient sample forms a sandwich between two specific antibodies to CRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon

the addition of the TMB substrate, the intensity of color is proportional to the concentration of CRP in the samples. A standard curve is prepared relating color intensity to the concentration of the CRP.

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	12x8x1
CRP Standard: 6 vials (ready to use)	0.25ml
CRP Conjugate Reagent: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Sample Diluent	50 ml
20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8 C.

Keep microwells sealed in a dry bag with desiccants.

The reagents are stable until expiration of the kit.

Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level

	2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984. 2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that standards, control and serum samples be run in duplicate. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8 C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENTS PREPARATION	1X Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Prior to assay, allow reagents to stand at room temperature (20-25C). Gently mix all reagents before use. Place the desired number of coated strips into the holder Dilute patient samples and controls 1:100 by adding 5 µl of samples to 495 µl of sample Diluent (STANDARDS ARE READY TO USE). Dispense 10 L of standard, diluted samples and controls into the appropriate wells Add 100 l of conjugate reagent to all wells. Tap the holder

to remove air bubbles from the liquid and mix well.
Incubate for 60 minutes at room temperature (20-25C).
Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbent paper towels.
Add 100 l of TMB substrate to all wells.
Incubate for 15 minutes at room temperature.
Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution.
Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:
Check CRP standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
To construct the standard curve, plot the absorbance for the CRP standards (vertical axis) versus the CRP standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
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.
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.

Example of a standard Curve

	OD 450 nm	Conc. mg/L
Std 1	0.011	0
Std 2	0.168	0.005
Std 3	0.362	0.01
Std 4	1.000	0.025
Std 5	1.682	0.05
Std 6	2.399	0.1

9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

LIMITATIONS OF THE TEST

The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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: the CRP level in normal human serum ranges from 0.2 to 10 mg/L, where 90% of apparently healthy individuals have CRP levels 10 mg/L.

PERFORMANCE CHARACTERISTICS

Precision

Intra-Assay

Serum	No. of Replicates	Mean (mg/L)	Standard	Coefficient of
Serum	No. of Replicates	Mean (mg/L)	Deviation	Variation (%)
1	24	0.60	0.04	7.16
2	24	4.25	0.25	5.99
3	24	9.48	0.47	4.95

Inter-Assay

Serum	No. of Replicates	Mean (mg/L)	Standard Deviation	Coefficient of Variation (%)
1	16	0.72	0.07	9.29
2	16	4.48	0.22	5.00
3	16	9.69	0.39	4.04

Linearity

Three different patient samples were diluted with the "0" calibrator to 1:2, 1:4, 1:8. CRP values were assayed and results were corrected with the dilution factor. The results

of these dilution tests are as follows

Original Value	Original Value	Percentage of Recovery	Percentage of Recovery	Percentage of Recovery
Serum	(mg/L)	1/2	1/4	1/8
1	7.2	94%	97%	98%
2	2.4	90%	98%	101%
3	5.4	95%	104%	110%

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 24 times in the same run.

Serum	No. of Replicates	Mean (mg/L)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	24	0.0097	0.001504	0.000136

Spiking Recovery

Samples were spiked with known amounts of CRP and assayed spiked and unspiked in duplicate in the same assay. Results were as follows:

CRP (mg/L)	CRP Spiked (mg/L)	Expected Value (mg/L)	Measured Value (mg/L)	Recovery (%)
0.47	2	2.47	2.82	114%
0.47	0.5	0.97	1.07	110%
0.47	0.2	0.67	0.66	98%

REFERENCES

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.

Kindmark, C.O.: The Concentration of C-reactive protein in Sera from Healthy Individuals. Scand J Clin Lab Invest, 29: 407- 411, 1972.

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.

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.

Kushner, I., Rzewnicki, D.L.: The acute phase response: General aspects. Bailliere's Clinical Rheumatology 8: 513-530, 1994.

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.

Hedlund, P.: Clinical and experimental studies on C-reactive protein (acute phase protein). Thesis Acta Med Scand, 128 (Suppl, 361):1-71, 1961.

Hedlund, P.: The appearance of acute phase protein in various diseases. Acta Med Scand, 128, (Suppl,196): 579-601, 1947.

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.