Version 22-12/24

User's Manual

Human C-Reactive Protein (CRP) ELISA Kit

REF **IVDIA1000-FA**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

C-Reactive Protein (CRP) ELISA Kit is a quantitative solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human C-Reactive Protein in serum to aid in the follow up of rheumatic fever, inflammatory processes, myocar.

General Description

C-Reactive Protein (CRP) has been regarded as an acute-phase reactant in serum. It consists of five single subunits with a molecular weight of 23,000, each noncovalently linked and assembled as a cyclic pentamer with a molecular weight range from 110,000 to 140,000 CRP has been found to be present in increased amounts in serum of patients with a wide variety of diseases including infections by gram-positive and gram-negative organisms acute phase of rheumatoid arthritis, myocardial infarction, malignant tumors abdominal abscesses, peritonitis, and inflammation of the bile duct. Less consistently, CRP may be found in patients with Guillain-Barre Syndrome and multiple sclerosis: in active tuberculosis acute infectious hepatitis, and certain viral infections; in many other necrotic and inflammatory diseases: in burned patients; and after surgery trauma.

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. CRP levels rise in serum within hours of the onset of inflammation, reaching a peak during the acute stage and decreasing with the resolution of inflammation or trauma. The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimantation rate (ESR), which may also be influenced by physiological changes not associated with an inflammatory process. Current quantitation methods including nephelometry, latex agglutination, radial immunodiffusion have the general disadvantages accompany agglutination and precipitation techniques.

Principles of Testing

The C-Reactive Protein (CRP) ELISA Kit is a solid phase enzyme linked immunosorbent assay (ELISA). The wells are coated with specific antibodies directed toward human CRP. An aliquot of patient sample containing the endogenous patient CRP is incubated in the well with an enzyme conjugate of horseradish peroxidase. Enzyme conjugate forms sandwich complex with CRP bound to the well. The unbound conjugate is washed off with water. The amount of bound peroxidase is proportional to the concentration of the CRP present in the sample. Upon addition of TMB Substrate, the intensity of color developed is proportional to the concentration of CRP in the samples.

Reagents And Materials Provided

1. Micro-wells strips (96 wells): Anti-CRP antibodies coated wells. 8x12 strips
2. Sample Diluent or zero standard (50 mL): 1 bottle
3. Enzyme conjugate (11 mL): Anti-CRP antibody conjugated with horseradish peroxidase.
4. Reference Standard Set (0.3 mL each vial)

The concentrations are 5, 10, 25, 50 and 100 ng/mL calibrated against WHO1st IS 85/506.

Positive Control(0.3 mL) value as indicated on the vials.

5. TMB Solution (11 mL): Buffer solution containing hydrogen peroxide and TMB
6. Washing buffer Concentrate (100X) (10mL): Prepare working washing buffer solution by adding 10 mL washing buffer concentrate into 990 mL distilled water.
7. Stop Solution: 2 N HCl.
8. Well holder: For securing individual wells.

Materials Required But Not Supplied

1. Micro-well reader at 450 nm.
2. Pipetor with tips for 5, 10 µL, 50 and 100 µL
3. 1 L washing bottle.

Storage

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The unopened reagents are stable until expiration of the kit.
3. TMB Solution should be colorless; if the solution turns blue, it must be replaced.

Specimen Collection And Preparation

Collect blood aseptically by venipuncture, allow to clot. Separate the serum by centrifugation at room temperature, and store in sterile tubes. If sera cannot be assayed immediately, they can be stored at 2-8°C for a week or frozen at -20°C for up to 6 months. Repeated freezing and thawing is not recommended.

Do not store in self-defrosting freezer. Do not use hyperlipemic, hemolyzed, contaminated or heat inactivated sample as they may cause erroneous results.

Assay Procedure

Preparation for Assay

1. Before beginning the test, bring all samples and reagents to room temperature (24±3°C) and mix each gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results
3. Use new disposable tips for each sample.

Assay Procedure

1. Secure the desired number of coated wells in holder.
2. Prepare 1:100 dilutions of test samples by adding 5 µL of sample to 0.5 mL sample diluent in the separate tubes.
3. Dispense 10 µL of Standards or diluted serum samples, in duplicate into appropriate well.

4. Dispense 100 µL of enzyme conjugate into each well and mix for 5 seconds and incubated at room temperature for 60 minutes.
5. Remove mixture and rinse the wells 5 times with washing buffer solution. (300 µL/well/each rinse) (Be sure to wash the wells thoroughly and completely dry the wells. Improper wash may cause false positive results).
6. Dispense 100 µL of TMB Solution into each well. Mix for 5 seconds and incubated in the dark for 30 minutes.
7. Stop reaction by adding 50 µL of stop solution to each well and read at 450 nm with microwell reader against Blank well (only Solution A and Solution B).

Note:

1. It is very important to wash the microwells thoroughly and remove the last droplets of water to achieve the best results.
2. Pipet all reagents and samples into the bottom of well. Avoid scratching the well. Vortex-mixing or shaking of wells is not required.
3. Absorbance is function of the time and temperature of incubations. It is recommended to have all reagents and samples caps removed, all needed wells secured in holder and assigned. This will ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason, the size of the assay run should be limited. It is suggested to run no more than 20 patients with a set of Reference Standards in duplicate.

Quality Control

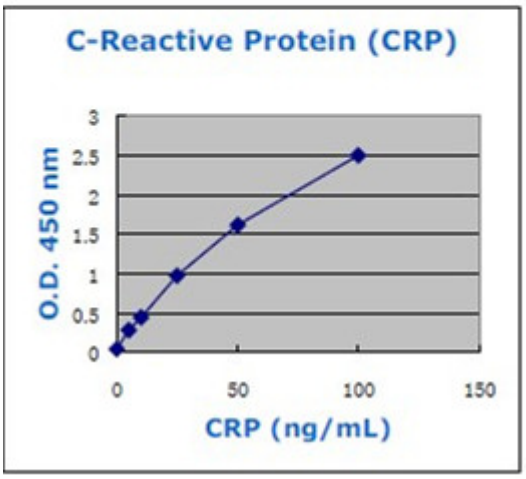
Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the Control.

Calculation

Any microwell reader capable of determining absorbance at 450 nm may be used. The CRP value of patient is obtained as follows:

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on linear graph paper.
2. Obtain the CRP values of samples by reference to the standard curve as follows and then times 100.

Well No.	Description (ng/mL)	Absorbance (450 nm)	CRP (ng/mL)
A 1	0	0.044	
A 2	5.0	0.282	
A 3	10.0	0.444	
A 4	25.0	0.973	
A 5	50.0	1.618	
A 6	100.0	2.501	
A 7	Patient A	1.490	40 x 100 = 40 v00



Reference Values

It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its environmental factor such as diet, climate etc.

The CRP concentration in normal serum samples (n=128) determined with CRP ELISA showed that 80

Precision

Intra-assay: Eleven samples each from three pooled sera were assayed in a single run. Inter-assay: Three pooled sera were assayed in duplicate in four days.

Sensitivity

0.35 ng/mL

Specificity

The following selected compounds were tested at levels approaching their highest possible concentrations allowable under the assay conditions. These were no slightest interference found.

Recovery

Recovery studies were performed by mixing an aliquot of pooled serum and CRP standard. The CRP values were measured and percentage of recovery determined.

Initial Value (ng/mL)	Expected Value (ng/mL)	Observed Value (ng/mL)	Recovery (%)
15	44	38	96
15	27	27	100
15	12	12	100

Precautions

1. It is designed for in vitro diagnostic use only.
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
3. Warning potential bio-hazardous material: The matrix of Negative and Positive controls is human serum. The serum found negative for HBsAg, HIV and HCV antibodies when tested with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HBsAg, HIV, HCV, or other infectious agents are absent, these reagents should be handled at Bio-safety level 2, as recommended for any potentially infectious human serum or blood specimen in the Center for Disease Control/National Institutes of Health Manual.

Limitations

For diagnostic purpose, the CRP values should be used as an adjunct to other data available to the physician.

References

1. Powell L.J., C-Reactive Protein- A review. AM. J. Med. Technol. 87:138-142, 1979.
2. Osmand, A.P., B. Friedenson, H.Gewurz, R.H. Painter, T. Hoffmann , and E. Shelton. Characterization of C-Reactive protein and the complement subcomponent C1t as homolo