Version 22-04/24

User's Manual

CRP (human) ELISA kit

REF **DEIA-NS2307-10**

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.

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PRODUCT INFORMATION

Intended Use

The CRP Enzyme-Linked Immunosorbent Assay (ELISA) kit is a colorimetric, competitive immunoassay kit for the quantitative determination of CRP in human serum and plasma. Please read the entire kit insert before performing this assay.

General Description

C-reactive protein (CRP) is a member of the pentraxin family of proteins, which also includes serum amyloid P component (SAP). Human CRP is comprised of five noncovalently associated subunits and forms a homopentameric structure. It is a widelyused biomarker for systemic inflammation and tissue injury. CRP specifically binds to phosphocholine (PCh) residues of polysaccharides on many microbial pathogens and of apoptotic or necrotic cell membranes in a Ca^{2+} -dependent manner. The PChbound CRP can be recognized by the C1q complex and efficiently initiate the activation of the complement system, which leads to the elimination of foreign pathogens. The binding of CRP to PCh on damaged cells can facilitate the clearance of apoptotic or necrotic host cells, contributing to restoration of normal structure and function of injured tissue. During myocardial infarction and ischemia/reperfusion injury, the same response occurring can lead to additional tissue damage resulting from complement activation. CRP also binds some nuclear constituents which do not contain PCh, such as histones and small nuclear ribonucleoproteins. The binding of CRP to Fc receptors FcγRI and FcγRIIa mediates the interaction of damaged cells or particles with phagocytic cells leading to phagocytosis of the cells or particles. The function of CRP in eliminating foreign pathogens and damaged cells through recruitment of the complement system and phagocytic cells deems CRP a very important molecule in the frontline of innate host defense.

In response to infection, cell damage or tissue injury, the serum CRP concentration may increase by up to 1000 fold. Elevated CRP levels have been reported in patients with infection, chronic inflammatory disorders, myocardial infarction, ischemia/reperfusion injury, atherosclerosis, cancer, pulmonary disorders, metabolic syndrome and depression.

Principles of Testing

1. Standards and samples are added to wells coated with a goat anti-mouse IgG antibody. A solution of CRP-Biotin conjugate and a solution of mouse monoclonal antibody to CRP are added sequentially. The plate is incubated at room temperature. During this incubation, the antibody binds the CRP in the sample or conjugate in a competitive manner. The plate is washed leaving only bound CRP.
2. A solution of streptavidin conjugated to horseradish peroxidase is added to each well to bind the biotinylated CRP. The plate is again incubated.
3. The plate is washed to remove excess HRP conjugate. TMB substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
4. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is inversely proportional to the level of CRP in the sample.

Reagents And Materials Provided

1. Goat anti-Mouse IgG Microtiter Plate, One Plate of 96 Wells

One plate using break-apart strips coated with goat antibody specific to mouse IgG.

2. CRP Conjugate (10×), 0.6 mL

One vial containing biotinylated C-reactive protein (CRP).

3. CRP Antibody (10×), 0.6 mL

One vial containing a mouse monoclonal antibody directed against C-reactive protein (CRP).

4. CRP Standard, 0.25 mL

One vial containing 6µg/mL C-reactive protein (CRP), purified from human origin.

5. Streptavidin-HRP, 20 mL

A solution of Streptavidin-conjugated Horseradish Peroxidase.

6. Assay Buffer 42, 100 mL

Tris buffered saline containing BSA, salts and detergents.

7. Wash Buffer Concentrate, 100 mL

20X Tris buffered saline containing detergents.

8. TMB Substrate, 25 mL

A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Protect from prolonged exposure to light.

9. Stop Solution 2, 10 mL

A 1N solution of hydrochloric acid in water. Keep tightly capped. **Caution: Caustic.**

10. CRP Assay Layout Sheet, 1 each

11. Plate Sealer, 3 each

Materials Required But Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipet for dispensing 50-200 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Lint-free paper for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

Storage

All components of this kit are stable at 4°C until the kit's expiration date except for the CRP Standard, CRP Antibody, and CRP Conjugate which must be stored at -20°C. Avoid repeated freezethaw cycles.

Specimen Collection And Preparation

This assay is suitable for the measurement of CRP in human serum and plasma. Prior to assay, frozen specimens should be brought to 4°C and centrifuged, and if necessary, filtered to remove residual debris. A minimum dilution of 1:4 in assay buffer is required to remove matrix interference with most serum or plasma specimens. Due to variation in samples, a different dilution may be required. Users must determine the optimal dilutions for their particular samples.

Serum Preparation

1. Collect whole blood in appropriate serum tubes.
2. Centrifuge at 1000 x g for 15 minutes at room temperature.
3. Remove serum to a clean plastic tube.
4. Divide serum into aliquots and store at or below -20°C, or use immediately in the assay.
5. Prior to assay, frozen serum should be brought to 4°C and centrifuged and if necessary, filtered to remove residual debris.
6. Avoid repeated freeze-thaw cycles.

Plasma Preparation

1. Collect whole blood in vacutainer tubes containing EDTA.
2. Centrifuge at 1000 x g for 15 minutes at 4°C.
3. Remove supernatant (plasma) to a clean plastic tube.
4. Divide plasma into aliquots and store at or below -20°C, or use immediately in the assay.
5. Prior to assay, frozen plasma should be brought to 4°C and centrifuged, and if necessary, filtered to remove residual debris.
6. Avoid repeated freeze-thaw cycles.

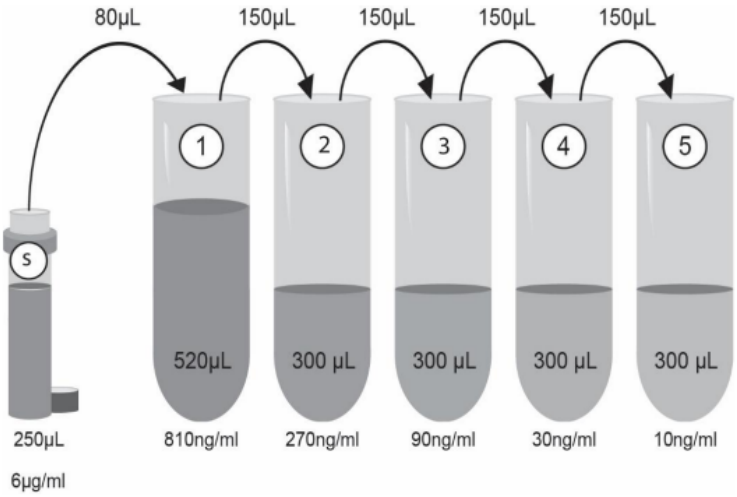
Reagent Preparation

1. Wash Buffer

Prepare the Wash Buffer by diluting 30 mL of the supplied concentrate with 570 mL of deionized water. The diluted wash buffer can be stored at room temperature for up to 3 months.

2. CRP Standard

Keep the CRP standard cooled on ice during preparation. Label five disposable 12 × 75 mm (or similar) polypropylene tubes #1 through #5. Add 520 µL of Assay Buffer 42 into tube #1 and 300 µL Assay Buffer into tubes #2 through #5. Add 80µl of standard stock into tube #1 and vortex gently. Serially dilute 150 µL of tube #1 standard to tubes #2 and vortex thoroughly. Continue this through tubes #3 through #5. Diluted standards should be used within 60 minutes of preparation. Discard any unused standard dilutions. Return stock solution of Human CRP (6µg/mL) standard to -20°C storage, avoid repeated freeze-thaw cycles.



3. CRP Conjugate

Prepare the 1× CRP conjugate by diluting 1:10 with supplied Assay Buffer 42 (i.e. 300 µL 10× CRP Conjugate in 2.7 mL Assay Buffer 42) Mix immediately by gentle vortexing. Return 10× CRP conjugate to -20°C Storage and avoid repeated freeze-thaw cycles. Do not store 1× diluted CRP conjugate.

4. CRP Antibody

The CRP antibody is supplied as a 10× concentrate. Dilute appropriate volume to 1x (i.e. 0.6 mL 10× stock into 5.4 mL supplied Assay Buffer) and store the unused 10× concentrate at -20°C.

Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the plate bag and seal. Store unused wells at 4°C.

1. Add 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Add 100 µL of the assay buffer into the B_0 (0 ng/mL standard) wells. Leave the Blank wells empty.
3. Add 100 µL of Standards #1 through #5 into the appropriate wells.
4. Add 100 µL of the samples into the appropriate wells.
5. Add 50 µL of the 1× conjugate into each well.
6. Add 50 µL of the 1× antibody into each well except NSB wells.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm*) at room temperature. See note.
8. Empty the contents of the wells and wash by adding full well volume, ~ 400 µL, of wash solution to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Add 200 µL of SA-HRP to each well except the Blank wells.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm*) at room temperature.
11. Wash as above (Step 8).
12. Add 200 µL of the TMB substrate solution into each well.
13. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm*) at room temperature.
14. Add 50 µL of Stop Solution 2 into each well.

15. Read the optical density at 450 nm.

***Note: The plate shaker speed was based on our plate shaker. The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

Calculation

Several options are available for the calculation of the concentration of CRP in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. If data reduction software is not readily available, the concentration of CRP can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Plot the Average Net OD versus concentration of CRP for the standards. Approximate a line through the points. The concentration of CRP in the unknown samples can be determined by interpolation.

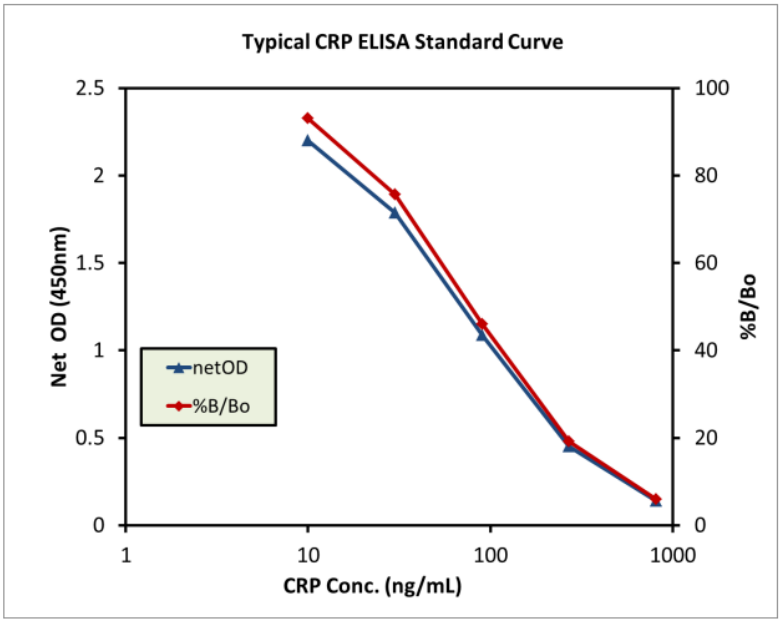
Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Typical Standard Curve

The results shown below are for illustration only and should not be used to calculate results.

Sample	CRP [ng/mL]	Average Net OD	%B/Bo
NSB (mean)	---	(0.045)	---
Bo	0.0	2.361	100
Std 1	810.0	0.140	5.9
Std 2	270.0	0.452	19.1
Std 3	90.0	1.087	46.0
Std 4	30.0	1.786	75.6
Std 5	10.0	2.199	93.1

Typical standard curves are shown below. These curves must not be used to calculate CRP concentrations; each user must run a standard curve for each assay.



Precision

The precision numbers listed below represent the percent coefficient of variation for the concentrations of CRP determined in these assays as calculated by a 4 parameter logistic curve fitting program.

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of CRP and running these samples multiple times (n=20) in the same assay.

ng/mL	%CV
416.2	3.4
114.2	4.5
26.9	13.5

Inter-assay precision was determined by measuring samples containing low, medium and high concentrations of CRP in multiple assays (n=5) over several days and by different operators.

ng/mL	%CV
415.3	3.6
115.0	11.3
31.9	10.4

Sensitivity

The sensitivity or limit of detection of the assay is 8.876 ng/mL, determined by interpolation at 2 standard deviations away from the mean signal of 20 replicates of zero standard.

Specificity

Human Serum Amyloid P Component (SAP) is approximately 50% homologous to CRP. Considering the possible cross reactivity between these two proteins, the cross reactivity to SAP was determined by running the competition assay with SAP and CRP conjugate. Comparing to the competitive binding of CRP, SAP had <0.015% competitive binding activity to anti-CRP antibody in this assay.

Linearity

The minimum required dilution for common human serum or plasma was determined by serially diluting specimens into the provided assay buffer and identifying the dilution at which linearity was observed. According to the below table, the minimum required dilution in both human serum and plasma is 1:4.

Dilution	Dil.Lin. Off Last Dil'n (%)	
	Human Serum	Human Plasma
Neat	---	---
1:4	100	100
1:8	101	111
1:16	93	105
1:32	92	99
1:64	101	110
1:128	101	---
Avg. Dil. Linearity	98	105

Recovery

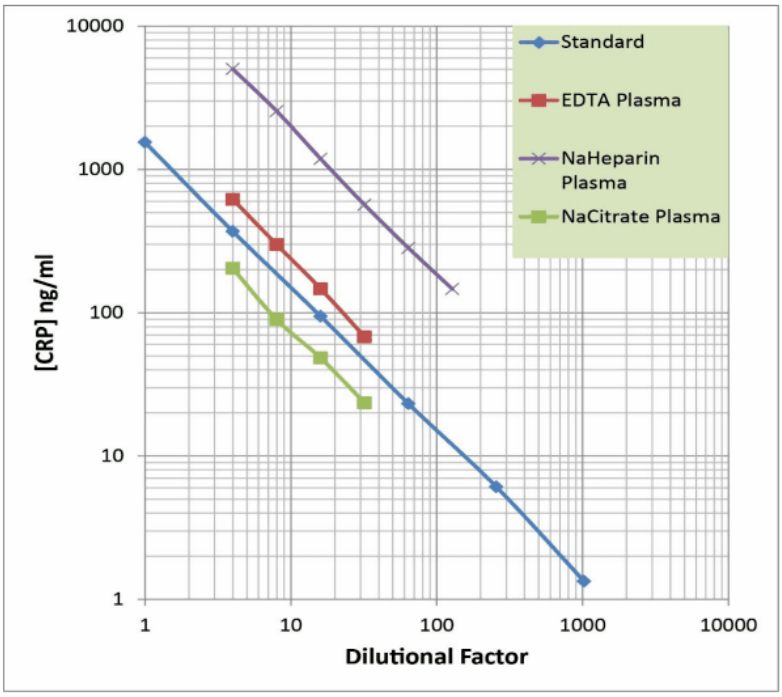
400 ng/mL and 100 ng/mL of purified native CRP protein was spiked into diluted human serum or plasma. Matrix background was subtracted from the spiked values and the recovery was compared to the recovery of identical spikes in assay buffer. The average percent recovery for each matrix is presented below. The CRP ELISA assay is capable of accurately detecting the CRP in serum or plasma without interference from the matrix. ***Please note that the endogenous level of the CRP in the samples along with the spiked CRP required dilution beyond the minimal recommended dilution in order to fall onto the standard curve.**

Sample Matrix	Dilution	Spike Concentration [ng/mL]	% Recovered
Human Serum	1:8	400	98
		100	94
Human Plasma	1:8	400	96
		100	97

Interferences

This kit is developed for measuring CRP concentration in the serum and plasma matrices of human origin. As plasma is usually prepared with addition of different anticoagulants, we analyzed the parallelism of human

plasmas prepared with addition of EDTA, Sodium Citrate, or Sodium Heparin as anticoagulants. The below graph showed the different anticoagulants didn't interfere with the CRP detection in the assay.



Precautions

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of CD. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.