

Human CRP ELISA KIT Cat. No. 1000

Kit Components, 96 tests	Cat #
Anti-human CRP coated strip plate (96 wells)	1001
CRP Std. A (0 ng/ml), or Sample Diluent, 16 ml	1002A
CRP Std B (100 ng/ml), 0.50 ml	1002B
CRP Std C (400 ng/ml), 0.50 ml	1002C
CRP Std D (1000 ng/ml), 0.50 ml	1002D
CRP Std E (4000 ng/ml), 0.50 ml	1002E
CRP Std F (10,000 ng/ml), 0.50 ml	1002F
Human CRP Low & High Control in a buffer, 0.5 ml each, (Lot sp. Conc given on the vial)	1002-CL1 1002-CL2
Anti-hCRP-HRP Conjugate, 0.3ml, Dilute 1:80 with assay buffer	1003
Assay Buffer, 40 ml	1000-AB
HRP substrate, Solution, 16 ml	TMB1000
Wash buffer (10X), 50 ml; dilute 1:10 with distilled water	W B 1 0 0 0
Stop solution, 6 ml	S T - 1 0 0 0
Instruction Manual	M-1000

Introduction

C-reactive protein (CRP) has been regarded as an acute phase reactant in serum. It consists of five single subunits, which noncovalently linked and assembled, as a cyclic pentamer with a mol. Wt. Range of 110-140 kDa. CRP has been found to be increased in serum of patients with a wide variety of diseases including infections by gram-positive and gram-negative bacteria, acute phase of rheumatoid arthritis, abdominal abscesses, inflammation of bile ducts (4), myocardial infarction, and malignant tumors. CRP may 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. Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is useful indicator of inflammatory processes. CRP levels rise in serum within hours of the onset of inflammation, reach a peak during the acute stage and decrease with resolution of inflammation trauma. The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate, which may also be influenced by physiological changes not associated with an inflammation process. Current quantification methods including latex agglutination, nephelometry, radial immunodiffusion have the general disadvantage accompany agglutination and precipitation techniques.

ADI's CRP ELISA provides is a very specific and sensitive assay for CRP.

4. LINEARITY

Three different patient samples (with original CRP concentration of 3662, 6120, 8800 ng/ml) were diluted (1:5, 1:25, and 1:50) with the assay buffer and their final CRP values determined. The samples showed excellent mean recoveries of about 94% (range 85-117%).

5. HIGH DOSE HOOK EFFECT

CRP concentrations of up to 160,000 ng/ml did not show any hook effect.

6. Correlative Study

The ADI's CRP ELISA kits were compared with Beckman Array System by analyzing 48 patient samples values from 0.37-0.339 ug/ml. The regression analyses showed good correlation (0.933) between these two methods.

7. Expected Normal Values

As for all assays, each laboratory must establish its normal values or reference ranges. In one study, we established:

	Males	Females	Combined
N	43	45	88
Age	17-87	12-79	12-87
Abs range	73-63,680 ng/ml	34-39240 ng/ml	34-63680 ng/ml
2.5 th percentile	132	139	135
50 th percentile	1197	1033	1104
97.5 th percentile	9710	6578	8910

8. Species reactivity

Human CRP kit has minimal crossreactivity with other species (mouse, rat, bovine etc). For this reason, ADI has developed CRP ELISA kits for rat (#1010) dog (#1020), rabbit (#1030) mouse (#1040), and monkey (#1050) CRP.

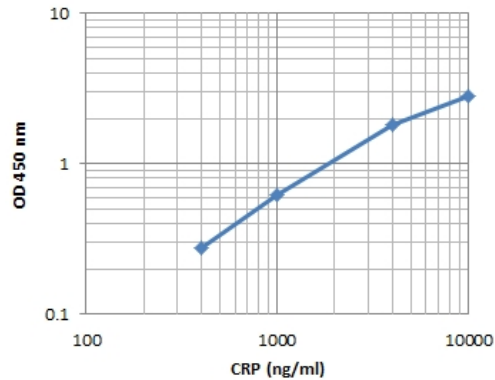
Published Citations of ADI's human CRP ELISA kit-(selective list)

Prio TK	2002	Expt. Gerontol. 37, 693-699	CRP ELISA on human urine from
Edwards DJ	2002		Med. & Science in Sports & Exercise. 34(5) Supplement 1:S180
Labarrere C	2002	Lancet 360, 1462-1467	CRP ELISA on human serum.
Bullo M	2003	Obes. Res., 11: 525 - 531	Human plasma.
Phillips T	2003	Med. & Science in Sports & Exercise. 35(12):2032-2037,	Human
Raio L	2003	Obstetrics & Gynecology 101, 1062-1063	human urine
Phillips T	2003	Med. & Science in Sports & Exercise. 35(12):2032-2037,	serum
Moe SM	2003	Kidney Intl. 63, 3, 1003-1011	CRP ELISA
Aggarwal A	2003	The Am. J. Cardiology, 91,1346-1349	CRP ELISA
Bruunsgaard H	2003	J. Nutr., 133: 1170 - 1173	plasma of human subjects.
Ghanim H	2004	Circulation 110: 1564 - 1571	CRP plasma in obese subjects
Obisesan TO	2004	Arterioscler. Thromb. Vasc. Biol., 24, 1874-1879,	CRP ELISA
Rotondi M	2004	Am. J. Transplantation 4, 9, 1466-1474	CRP ELISA
Khaothiar L	2004	JPEN J Parenter Enteral Nutr, 28: 410 - 415	CRP in human serum
Lee KT	2004	Cardiology, 2004, Vol. 102 Issue 3, p166-170,	human CRP ELISA
Tondeur MC	2004	Am. J. Clinical Nutrition, 80: 1436 - 1444	CRP plasma
Fusshoeller A	2004	Nephrol. Dial. Transplant., 19: 2101 - 2106	plasma CRP,
Fabbi P	2004	J. Laboratory Clinical Med., 143, 99-105	serum crp elisa
Cerchiatti LCA	2004	J. Pain Symptom Manage. 27(1):85-95.	CRP ELISA
Aggarwal A	2004	The Am. J. Cardiology, 93, 6-9	CRP ELISA
Blum A	2004	Am. J. Cardiology, 94, 1420-1423	CRP in human serum

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450 nm}
A1, A2	Std. A (0 ng/ml)	0.054
B1, B2	Std. B (100 ng/ml)	0.104
C1, C2	Std. C (400 ng/ml)	0.274
D1, D2	Std. D (1000 ng/ml)	0.620
E1, E2	Std. E (4000 ng/ml)	1.929
F1, F2	Std. F (10,000 ng/ml)	2.828
G1, G2	Sample 1	1.042

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Kit-spec-XL

A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate CRP concentrations. Read off the CRP concentrations of the control and patient samples directly from the standard curve. **DO NOT MULTIPLY THE SAMPLES VALUES BY 1:20 AS THIS HAS ALREADY BEEN TAKEN INTO ACCOUNT OF THE STANDARDS.** If samples were diluted more than 1:20 then the values should be multiplied by the dilution factor. Examples: A sample was diluted 1:40 then this values should be multiplied by 1:2 or a sample that was diluted 1:100 then the values be multiplied by 1:5.

For easy calculations, It is possible to re-state the values of the standards (1/20th of what is on the vial (e.g, 0, 20, 80, 200, 500 ng/ml) and apply dilution factor of the samples.

If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve. Sample dilution should be as explained above.

PRINCIPLE OF THE TEST

Human CRP ELISA kit is based on simultaneous binding of human CRP from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of CRP present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of CRP in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI CRP ELISA kit is intended for *in vitro* research use only. The reagents contain proclin-100 (0.1%) as preservative; necessary care should be taken when disposing solutions. The stds./controls sera contain human serum that has been shown to be negative for HbsAg, HCV, and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled at biosafety level 2, as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH Manual, "Biosafety in microbiological and biomedical laboratories, 1984".

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum.. Do not add azide or other preservatives. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. This kit has not been optimized for plasma, urine, or saliva culture medium. Users must optimized the assa.

REAGENTS PREPARATION FOR THE ASSAY

Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml). Store at 4°C.

Samples. Before use, dilute 1:20 with Std A (10 ul sample in 190 ul of Std A).. It is possible to take less for dilution, but it may increase error. It is possible to use, normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

Dilute enzyme conjugate 1:80 (eg; 25 ul of HRP in 2 ml assay buffer). For whole plate, take 150 ul conjugate in 12 ml of assay buffer.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions. After opening the kit components, the shelf life is approx. 2 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **Dilute wash buffer 1:10** with water. **Dilute HRP conjugate 1:80** in assay buffer. Label or mark the microtiter well strips to be used on the plate.

1. Dilute serum samples **1:20** using **std A** or sample diluent. Do not dilute standards or controls. Pipet **20 ul stds** and diluted samples into appropriate wells.
2. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used for sample dilution. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipet.
3. Pipet **200 ul** assay buffer into each well using multichannel pipette. Cover the plate and incubate on a plate shaker (approx. 200 rpm) for **30 minutes** at room temperature. Failure to shake the plate will reduce the color development.
4. Aspirate and **wash the wells 3 times** with wash buffer (300 ul/well/wash). We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. Pipet **100 ul of diluted Ab-enzyme conjugate** into each well. **Mix gently for 5-10 seconds**. Cover the plate and incubate on a plate shaker (approx. 200 rpm) for **15 minutes** at room temperature.
6. Aspirate and **wash the wells 3 times** with wash buffer (same as in step 4).
7. Dispense **100 ul TMB substrate solution per well**. Mix gently. Cover the plate and incubate on a plate shaker for **15 minutes** at room temp. incubation time may be + 5 min so as to get maximum $A_{450} < 3.00$. Blue color develops in standards and positive wells.
8. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 8. Mix gently for 5-10 seconds to make ensure even color distribution. Blue color turns yellow.
9. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 1 hr after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Our recommended dilution of the samples is 1:20 that should bring most samples within the detection range. Samples containing CRP more than highest standards (10,000 ng/ml CRP) should be diluted further beyond the initial dilution of 1:20 (e.g., 1:20 samples diluted another 1:5 or a total of 1:100). The results obtained should be multiplied by the appropriate 2nd dilution factor, i.e 1:5. It is possible to use, normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

QUALITY CONTROL

Standards and controls must perform as stated in the manual. If controls are out of range then the test must be repeated.

SPECIFICITY

The specificity of CRP ELISA kit was determined by measuring interference from high concentrations of various relevant compounds. There was no appreciable interference from high concentration of albumin of IgG..

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT- Based on sixteen replicate determinations of the zero standard, the minimum CRP concentration detectable using this assay is 10 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Three serum samples (mean CRP concentrations 205.8, 769.2, 8437.8 ng/ml) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 12, 5, and 6.3, respectively.

Inter-assay precision: Three serum samples (227, 1022.2, 8791.8 ng/ml) were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (9.9, 9.5, and 7.8% CV).

3. RECOVERY-A known amount of hCRP was added to three patient sera (with original CRP concentrations of 263, 760, 5546 ng/ml) and the total CRP concentration measured. The assay showed excellent mean recoveries of about 94% (range 92-115%).