Human CRP ELISA Kit

For the quantitative determination of human C Reactive Protein (CRP) concentrations in serum and plasma.

Catalogue Number: EL10022

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE

This Human CRP ELISA kit is to be used for the *in vitro* quantitative determination of human c reactive protein (CRP) concentrations in serum and plasma. This kit is intended FOR LABORATORY RESEARCH USE only and not for use in diagnostic or therapeutic procedures.

INTRODUCTION

C-Reactive Protein (CRP) is an acute phase protein, originally identified and named for its ability to precipitate the C-polysaccharide of pneumococcus in the presence of calcium[1]. It is the prototypic acute phase reactant whose presence in plasma or serum serves as a useful laboratory indicator of systemic inflammatory disease. Normally, CRP in human biological fluids is present in trace amounts (0.07-8.00 mg/L, median 0.6 mg/L). Stimulated by certain cytokines (IL-1α, IL-1β, TNF-α and β, and indirectly by IL-6)[2,3], its sythesis by hepatocytes enhanced dramatically. Thus during the acute phase response CRP's serum concentration can increase up to 1000-fold within a few hours[3].

Human CRP is a kind of nonimmunoglobulin serum substance, a heat labile β-globulin. It is classified in a superfamily of proteins termed pentaxins or pentraxins: cyclic, non-glycosylated structures composed of five apparently identical globular non-covalently linked subunits aggregated symmetrically. Each subunit is 23.05 kD (206 amino acids), with a total molecular weight of 117.5 kDa, and consists of 14 anti-parallel β-strands arranged in two β-sheets[4,7].

Among acute phase proteins, CRP is a fast-reacting, sensitive and the most easily measured one. It has a rapid response time, short half life and large incremental change and its catabolism is not affected by the type of inflammation. Moreover, its normalization can monitor the cure process from the disease, hence it has the largest data base of disease-released change.

CRP can be used as an early and pre-clinical marker of inflammation. Especially in situations where microbiological diagnosis is difficult or too slow in the clinical context, CRP measurements can be used to infer the presence of bacterial infection[5]. Following acute tissue damage or during the course of infectious and non-infectious diseases, hepatic synthesis of CRP dramatically increases its plasma concentration from 0.58 (range 0.06-8.0) mg/L to 100-500 mg/L within 24-48 hours. Typically, mild elevations of CRP (10-40 mg/L)[6,8] are seen in a variety of inflammatory conditions such as viral infections, rheumatoid arthritis, Crohn's disease, post-transplant rejections, infant septic coxitis (arthritis of the hip joint), and neoplasia, whereas more marked rises (CRP 40->500 mg/L) are usual seen in acute and severe bacterial infections such as acute, severe pancreatitis and appenditis, bacterial or purulent meningitis, aspirition pneumonia, sequela-prone osteomyelitis, osteoarthritis, giant cell arthritis, septicaemia, systemic vasculitis, acute sinusitis, acute otitis media, infected urinary tract obstruction, extensive trauma, fractures, burns, infectious post-operative complications and acute myocardial infarction[6,8,9]. Combined with IL-6, CRP seems to be a valuable parameter in the early diagnosis of neonatal infections.

Serum amyloid A (SAA) is another major acute phase protein whose response is highly correlated with that of CRP (r=0.75-0.88, P=0.0001)[10,11]. Both CRP and SAA respond sensitively to several stimuli with elevated serum levels including surgical trauma, acute infection and allograft rejection, and show almost similar kinetics in many diseases processes. But they differ in certain responses, e.g., the reactivity and sensitivity of SAA is higher than CRP in viral infection, patients on steroid therapy, chronic allograft rejection. So the combined use of CRP and SAA may distinguish viral from bacterial infection, and may distinguish allograft rejection from infection. Meanwhile, CRP proved more useful than SAA to predict cardiovascular events and monitor process of diseases and the effects of some treatments.

PRINCIPLE OF THE ASSAY

This CRP enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for CRP. Standards or samples are then added to the appropriate microtiter plate wells and incubated. CRP if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound CRP and other components of sample. In order to quantitate the amount of CRP present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated antibody specific for CRP is added to each well to "sandwich" the CRP immobilized during the second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain CRP and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of CRP in the samples, this kit standard (ready-to use) is assayed at the same time as the samples (diluted if necessary with Sample Diluent). This allows the operator to produce a standard curve of Optical Density (O.D.) versus CRP concentration (μg/mL). The concentration of CRP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

	96 tests
1. **CRP MICROTITER PLATE** (Part 30025) Pre-coated with anti-human CRP monoclonal antibody.	***96 wells***
2. **CRP CONJUGATE** (Part 30026) Anti-human CRP antibody conjugated to horseradish peroxidase with preservative. *Ready-to-use*.	***12 mL***
3. **CRP STANDARD – 5 μg/mL** (Part 30095) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
4. **CRP STANDARD – 2 μg/mL** (Part 30096) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
5. **CRP STANDARD – 1 μg/mL** (Part 30097) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
6. **CRP STANDARD – 0.5 μg/mL** (Part 30098) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
7. **CRP STANDARD – 0.25 μg/mL** (Part 30099) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
8. **CRP STANDARD – 0.125 μg/mL** (Part 30100) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
9. **CRP STANDARD – 0.062 μg/mL** (Part 30101) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
10. **CRP STANDARD - 0 μg/mL** (Part 30102) Liquid human CRP in an animal serum with preservative that is ready-to-use.	***1 mL***
11. **WASH BUFFER (20X)** (Part 30005) 20-fold concentrated solution of buffered surfactant.	***60 mL***
12. **SUBSTRATE A** (Part 30006) Buffered solution with H_2O_2.	***10 mL***
13. **SUBSTRATE B** (Part 30007) Buffered solution with TMB.	***10 mL***
14. **STOP SOLUTION** (Part 30008) 2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!	***14 mL***
15. **SAMPLE DILUENT** (Part 30003) Animal serum with 0.1% Sodium Azide (NaN_3).	***15 mL***

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL required for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

   a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
   b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C), and serum extracted.
   c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate. *This CRP ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin*.

   - Avoid grossly hemolytic, lipidic or turbid samples.
   - Serum, plasma, cell culture supernatant, and urine samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. <u>Avoid freeze-thaw cycles.</u>
   - When performing the assay, slowly bring samples to room temperature.
   - It is recommended that all samples be assayed in duplicate.
   - DO NOT USE HEAT-TREATED SPECIMENS.

2. DILUTION PROCEDURES

   We recommend a 1:50 dilution of samples that are out of assay range:

   5 μL sample + 245 μL of Sample Diluent

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **CRP Standard:** no preparation required, all standards are ready-to-use.

ASSAY PROCEDURE

1. Prepare Wash Buffer before starting assay procedure. *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents		Wells	Contents	
1A, 1B	Standard 1 – **0 μg/mL**	(S1)	**2A, 2B**	Standard 5 – **0.5 μg/mL**	(S5)
1C, 1D	Standard 2 – **0.0625 μg/mL**	(S2)	**2C, 2D**	Standard 6 – **1.0 μg/mL**	(S6)
1E, 1F	Standard 3 – **0.125 μg/mL**	(S3)	**2E, 2F**	Standard 7 – **2.0 μg/mL**	(S7)
1G, 1H	Standard 4 – 0.25 μ**g/mL**	(S4)		Standard 8 – **5.0 μg/mL**	(S8)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	3	7	11	15	19	23	27	31	35	39
B	S1	S5	3	7	11	15	19	23	27	31	35	39
C	S2	S6	4	8	12	16	20	24	28	32	36	40
D	S2	S6	4	8	12	16	20	24	28	32	36	40
E	S3	S7	5	9	13	17	21	25	29	33	37	41
F	S3	S7	5	9	13	17	21	25	29	33	37	41
G	S4	S8	6	10	14	18	22	26	30	34	38	42
H	S4	S8	6	10	14	18	22	26	30	34	38	42

2. Add 100 μL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate. Mix, by gently tapping the plate. Cover and incubate for 30 minutes at 37^oC.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper

towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Dispense two drops or 100μL of conjugate to each well. Cover and incubate for 30 minutes at 37°C.

5. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

6. Repeat wash procedure as described in Step 3.

7. Add 100 μL Substrate Solution to each well. Cover and incubate for 15 minutes at 37°C.

8. Add 100 μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of CRP in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding CRP concentration (μg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 μg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

To determine the amount of CRP in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding CRP concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor (x 50).

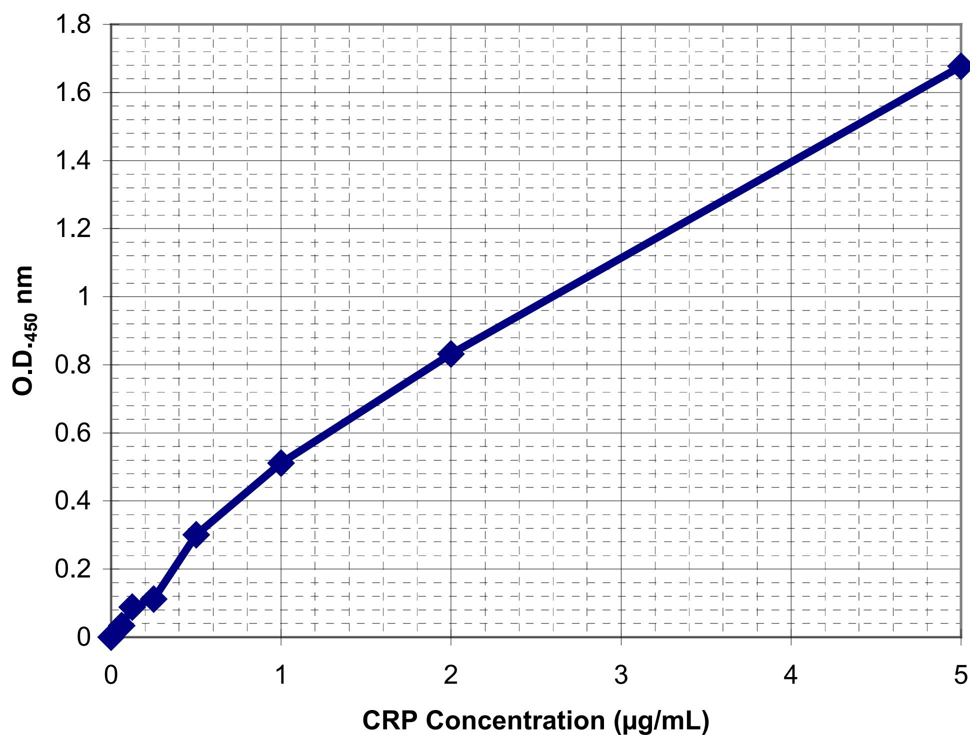
TYPICAL DATA

Results of a typical standard run of a CRP ELISA are shown below. Any variation in sample diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve

EXAMPLE

The following data was obtained for a standard curve.

Standard (μg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.009, 0.004	0.006	0
0.0625	0.043, 0.037	0.040	0.034
0.125	0.096, 0.092	0.094	0.088
0.25	0.172, 0.164	0.118	0.112
0.5	0.323, 0.292	0.907	0.301
1.0	0.522, 0.512	0.517	0.511
2.0	0.845, 0.831	0.838	0.832
5.0	1.716, 1.650	1.683	1.677



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION
   To determine within-run precision, three different samples of known concentration were assayed by replicates of 20 in 1 assay.

Sample	1	2	3
n	20	20	20
Mean (μg/mL)	0.719	1.880	2.0
Standard Deviation (μg/mL)	0.05	0.076	0.11
Coefficient of Variation (%)	*6.9*	*4.0*	*5.5*

2. INTER-ASSAY PRECISION
   To determine between-run precision, three different samples of known concentration were assayed by replicates on 20 different assays.

	Calibrator Diluent II assay		
Sample	1	2	3
n	20	20	20
Mean (μg/mL)	0.663	1.878	2.12
Standard Deviation (μg/mL)	0.045	0.09	0.106
Coefficient of Variation (%)	*6.7*	*4.8*	*5.0*

3. SENSITIVITY
   The minimum detectable dose of CRP was determined by adding two standard deviations to the mean optical density value of the 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of CRP using a standard curve is 0.03 μg/mL.

4. SPECIFICITY
   No cross reaction observed.

5. CALIBRATION
   This immunoassay is calibrated against CRM No. 470-CAP/IFCC.

6. EXPECTED NORMAL VALUES
   Biological samples from apparently healthy, normal individuals were collected and the average CRP concentration measured. Serum samples (n=40) averaged 0.32 μg/mL (range: 0.25-1.5 μg/mL). Plasma samples collected with EDTA, heparin, and citrate (n=20) averaged 0.62 μg/mL (range: 0.25-1.75 μg/mL).

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