High Sensitivity Human CRP ELISA Kit

For the quantitative determination of human C Reactive Protein (CRP) concentrations

Catalogue Number: EL10022hs

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. This kit is intended **FOR LABORATORY RESEARCH USE** only, therefore, should not be used in any diagnostic or therapeutic procedures.

INTRODUCTION

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.

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. 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 TNF-β, and indirectly by IL-6), its synthesis by hepatocytes enhanced dramatically. During the acute phase response, CRP concentration can increase up to 1000-fold within a few hours.

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. Following acute tissue damage or during the course of infectious and non-infectious conditions, hepatic synthesis of CRP dramatically increases. Typically, mild elevations of CRP are seen in a variety of inflammatory conditions. Serum amyloid A (SAA) is another major acute phase protein whose response is highly correlated with that of CRP. Both CRP and SAA respond sensitively to several stimuli, but they differ in certain responses.

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 an 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 (mg/L). 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 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **CRP MICROTITER PLATE** (Part EL22hs-1) ***96 wells***
   Pre-coated with anti-human CRP monoclonal antibody.
2. **CRP CONJUGATE** (Part EL22hs-2) ***12mL***
   Anti-human CRP antibody conjugated to horseradish peroxidase with preservative. *Ready-to-use*.
3. **CRP STANDARD** 0 mg/L (Part EL22hs-3) ***1mL***
   Buffered protein base with preservative.
4. **CRP STANDARD** 0.005 mg/L (Part EL22hs-4) ***1mL***
   0.005 mg/L CRP in a buffered protein base with preservative.
5. **CRP STANDARD** 0.010 mg/L (Part EL22hs-5) ***1mL***
   0.010 mg/L CRP in a buffered protein base with preservative.
6. **CRP STANDARD** 0.025 mg/L (Part EL22hs-6) ***1mL***
   0.025 mg/L CRP in a buffered protein base with preservative.
7. **CRP STANDARD** 0.050 mg/L (Part EL22hs-7) ***1mL***
   0.050 mg/L CRP in a buffered protein base with preservative.
8. **CRP STANDARD** 0.100 mg/L (Part EL22hs-8) ***1mL***
   0.100μg/mL CRP in a buffered protein base with preservative.
9. **TMB SUBSTRATE** (Part EL22hs-9) ***11 mL***
   Ready to use TMB substrate
10. **STOP SOLUTION** (Part EL22hs-10) ***11 mL***
    1N hydrochloric acid (1N HCl). Caution: Caustic Material!
11. **CRP SAMPLE DILUENT** (Part EL22hs-11) ***50 mL***
    PBS buffer contains BSA with preservative.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips. 5-10μL, 100μL and 500μL are required for running the assay.
2. Distilled or deionized water.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Microtiter plate reader (450 nm ± 2nm)
7. Automatic microtiter plate washer or squirt bottle.
8. Sodium hypochlorite solution, 5.25% (household liquid bleach).
9. Plastic plate cover.
10. Disposable gloves.
11. 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 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. 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*.

SAMPLE PREPARATION

1. <u>**COLLECTION, HANDLING, AND STORAGE**</u>

   Serum sample preparation: 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.

   - Samples which cannot be assayed within 24 hours of collection should be frozen at -20ºC or lower, and will be stable for up to six months.
   - When performing the assay, slowly bring samples to room temperature.
   - It is recommended that all samples be assayed in duplicate.

2. <u>**DILUTION PROCEDURE**</u>

   Samples generally should be 1:100 diluted with CRP SAMPLE DILUENT prior to assay to identify out of normal range samples (CRP level higher than 10 mg/L). To make the dilution, agitate the sample, add 5µL sample to 495µL CRP SAMPLE DILUENT and mix well before assay.

   For samples with expected concentrations over 10 mg/L, dilute the sample further by adding 20µL of the 1:100 diluted sample into 180µL CRP SAMPLE DILUENT. If samples are still over range, dilute samples further with CRP SAMPLE DILUENT, and repeat the assay.

   For accurate measurement of CRP values at very low levels, such as in Cord Serum, the samples should be tested at low dilutions such as 1:10 dilution and undiluted.

   <u>**PLEASE DO NOT DILUTE STANDARDS.**</u>

ASSAY PROCEDURE

1. *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.* <u>**PLEASE DO NOT DILUTE STANDARDS.**</u>

Wells	Contents		Wells	Contents	
1A, 1B	Standard 1 – **0 mg/L**	(S1)	**2A, 2B**	Standard 5 – **0.050 mg/L**	(S5)
1C, 1D	Standard 2 – **0.005 mg/L**	(S2)	**2C, 2D**	Standard 6 – **0.100 mg/L**	(S6)
1E, 1F	Standard 3 – **0.010 mg/L**	(S3)	**2E-12H**	Samples	
1G, 1H	Standard 4 – **0.025 mg/L**	(S4)			

	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	1	5	9	13	17	21	25	29	33	37	41
F	S3	1	5	9	13	17	21	25	29	33	37	41
G	S4	2	6	10	14	18	22	26	30	34	38	42
H	S4	2	6	10	14	18	22	26	30	34	38	42

2. Add 10 µL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate.

3. Dispense 100µL of conjugate to each well. Thoroughly mix for 30-60 seconds.

4. Cover plate and incubate for 45 minutes at Room Temperature (18-25°C).

5. 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 distilled or deionized water 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 distilled or deionized water. 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.*

5. Add 100 µL TMB Substrate to each well. Cover and incubate for 10-15 minutes at Room Temperature.

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

7. 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 (mg/L) on the horizontal (X) axis.

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 mg/L) 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.**

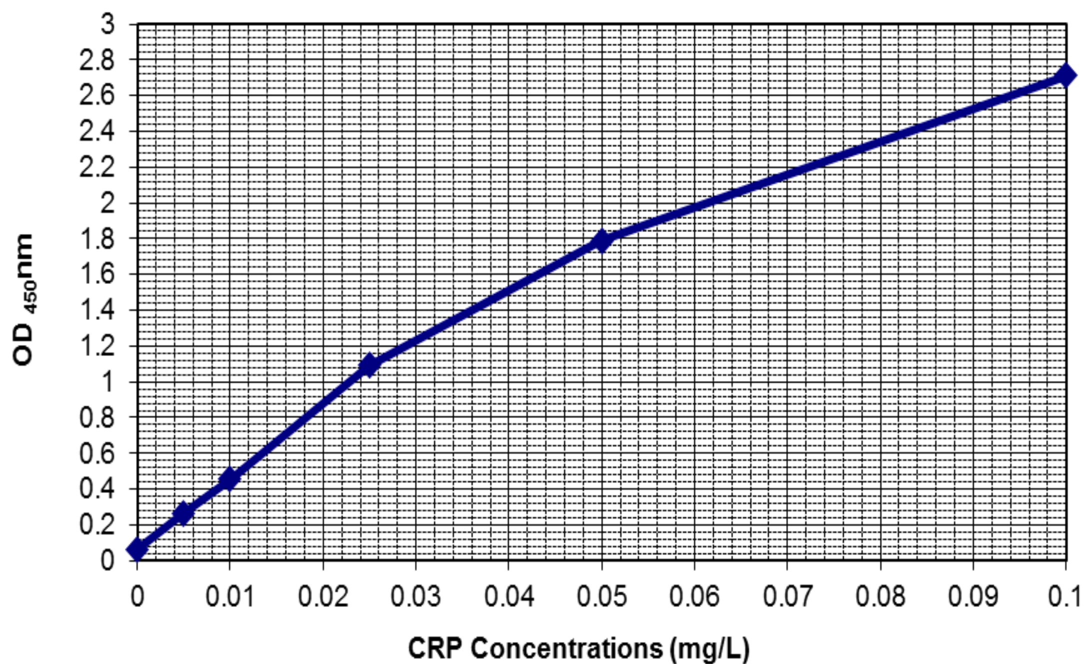
TYPICAL DATA

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 (mg/L)	Mean OD450
0.00	0.066
0.0	0.264
0.005	0.457
0.010	1.092
0.050	1.788
0.100	2.710



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 (mg/L)	0.719	1.880	2.0
Standard Deviation (mg/L)	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.

Sample	1	2	3
N	20	20	20
Mean (mg/L)	0.663	1.878	2.12
Standard Deviation (mg/L)	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 0.03mg/L.

4. **SPECIFICITY**
No cross reaction with other substances was observed.

5. **SAMPLE VALUES**
CRP levels are significantly affected by pro-inflammatory variables. Healthy individuals are expected to have CRP values below 10 mg/L. The sample values below are based on publications. It is recommended that each laboratory establish its own normal range based on the patient population.

Cord serum: CRP levels at birth are low. Cord blood normally has low CRP concentrations (0.01 - 0.35 mg/L), but in intra-uterine infection, levels may increase significantly.

Newborn serum: Postnatal age influences CRP values. According to a recent investigation, CRP mean values are significantly higher at 48 hours (4.10 mg/L) than at both 24 (2.30 mg/L) and 12 hours of life (0.80 mg/L).

Adult serum: 0.068 to 8.2 mg/L.

6. **CALIBRATION**
This immunoassay is calibrated against the WHO 1st International Standard for C Reactive Protein: 85/506.

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