High Sensitivity Human CRP ELISA Kit

Catalog No. EA200010

Principle of the Assay

C-Reactive Protein (CRP), also known as Pentraxin 1, is a non-glycosylated protein in the Pentraxin family. CRP assembles non-covalently into a 110-120 kDa cyclical pentamer. It functions as a sensor and activator of the innate immune response, and is involved in several host defense related functions. CRP is a major acute-phase protein which can be used as a general screening aid for inflammatory diseases, infections, and neoplastic diseases. The level of this protein in plasma increases greatly during acute phase response to tissue injury, infection, or other inflammatory stimuli. CRP has been considered as the most commonly used inflammatory biomarker. In addition, measurement of CRP by high-sensitivity CRP assays may add to the predictive value of other cardiac markers.

This sandwich ELISA is used to measure human CRP in serum, EDTA-plasma, urine and other biological fluid samples. Microtitration wells coated with anti-human CRP capture antibody are exposed to test specimens. The CRP antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured CRP antigen is then reacted with a biotinylated human CRP detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of CRP present in a sample.

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

Description	Quantity
CRP Antibody Coated 96-well Plate in foil pouch with desiccant	1
Native human CRP Standard (250ng/mL)	0.1 mL
Biotinylated CRP Detection Antibody (100x)	120 µL
Streptavidin Conjugated Horseradish Peroxidase (100x)	120 µL
Assay Buffer	30 mL
Sample Diluent	60 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

Additional Materials not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL to 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 15mL.
5. Laboratory glassware consisting of 100 mL beaker, 100 mL and 1 L graduated cylinders, 5 mL, 10mL and 25 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

Storage and Stability

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips should be returned to their original foil pouch along with the desiccant. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

Indications of Deterioration

The human CRP Assay kit may be considered to have deteriorated if:

1. Reagents are visibly cloudy.
2. The Substrate Solution turns blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Precautions

1. The reagents supplied in this kit are for ***Research use only***.

2. All blood products should be treated as potentially infectious.

2. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

3. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in case of contact with skin or eyes. Obtain medical attention if necessary.

Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.

2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents.
4. Some reagents in the CRP ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
6. Warm up the foil bag to room temperature before opening.
7. All reagents should be added to the plate in the same order.
8. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
9. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow manufacturer's recommendations.
10. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

Sample Collection and Storage

The Human CRP ELISA is intended for use with cell culture supernatants, serum, EDTA-plasma and other biological fluids. The sample should be tested as soon as possible. However, if the sample needs storage, it should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Frozen samples that have been thawed should be thoroughly mixed before testing.

Cell Culture Supernatant: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma: Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Urine: Collect the first urine of the day (mid-stream) aseptically. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rinse Cycle

Aspirate each well and wash. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser, or automatic plate washer. Complete removal of liquid at each step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

For the rinse cycle, the machine should be set to three washes for the first two rinse cycles and five washes for final rinse cycle.

Alternatively, the following manual system may be employed:

1. Discard or aspirate well contents.
2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle or multichannel pipette.
3. Discard or aspirate fluids.
4. Repeat steps 2 and 3, two times (Repeat steps 2 and 3 four times in final rinse cycle of the assay).

Preparation for the Assay

1. *Standard preparation*: Prepare protein standard 1 by diluting 10µL of standard stock into 490 µL (1:50 dilution) of sample diluent. This will give a final concentration of 5000 pg/mL as shown in Table 1. Make 2x serial dilution of Standard 1 using sample diluent to generate a standard concentration range of 78.13 to 5000 pg/mL.

Table 1: Human CRP Standard Curve Generation

Standard Number	Concentration of CRP (pg/mL)	CRP Standard (µL)	Sample Diluent (µL)
1	5000	10	490
2	2500	250 of #1	250
3	1250	250 of #2	250
4	625	250 of #3	250
5	312.5	250 of #4	250
6	156.25	250 of #5	250
7	78.13	250 of #6	250
8	0		250

2. *Sample preparation*: CRP concentration must be estimated prior to performing the full experiment by testing a serially diluted representative sample using assay buffer. Select an optimal dilution level such that the final target protein concentration falls near the middle of the assay linear dynamic range. If sample need more than 200-fold dilution, two or more dilution steps are suggested. Following is an example for 1000-fold dilution:

- Step 1 (20-fold dilution): 5 µL of sample + 95 µL of assay buffer
- Step 2 (50-fold dilution): 5 µL of 20-fold diluted sample + 245 µL of assay buffer

3. *Detection antibody preparation:* dilute the concentrated biotin conjugated detection antibody 1:100 using assay buffer.

4. *SA-HRP preparation*: dilute the concentrated streptavidin HRP conjugate 1:100 using assay buffer.

5. *Wash buffer*: Dilute 20x Wash Buffer Concentrate to 1x with distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

Assay Procedure

Note: All standards, controls and samples should be tested in duplicate.

1. Warm up kit to room temperature (18-25°C).

2. Select sufficient microplate strips to accommodate all test samples, controls and reagent blank. Fit the strips into the holding frame.

3. Add 100 µL of each standard and sample into appropriate wells. Note: Depending on the CRP concentration of your sample, dilution using sample diluent may be needed. If the sample CRP concentration is not known, you need to titrate the original sample.

4. Incubate for 2 hours at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

6. Add 100 µL of working concentration detection antibody into each well and incubate for 1.5 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

8. Add 100 µL of working concentration Streptavidin HRP conjugate into each well and incubate for 25 minutes at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

11. Wash the microtitration plate 5 times as described in the Rinse Cycle section.

12. Add 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 20-25 minutes.

13. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

14. Read the absorbance values at 450 nm using a microplate reader. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation of Results

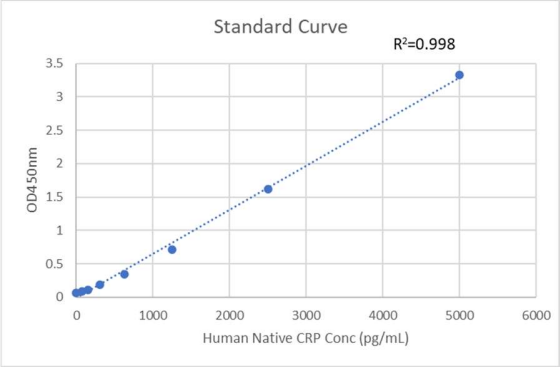
Average the duplicate readings for each standard and sample, and subtract the average zero standard optical density (O.D.).
A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

Table 2. Example Data at 450nm.

Standards	450 nm absorbance
Standard 1 (5000 pg/mL)	3.3236
Standard 2 (2500 pg/mL)	1.6225
Standard 3 (1250 pg/mL)	0.7172
Standard 4 (625 pg/mL)	0.3386
Standard 5 (312.5 pg/mL)	0.1823
Standard 6 (156.25 pg/mL)	0.1136
Standard 7 (78.13 pg/mL)	0.0839
Standard 8 (0 pg/mL)	0.0622

Typical Human CRP ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



Note: This standard curve is only an example and should not be used to generate any results.

Performance Characteristics

1. **Recovery**
The recovery of human CRP spiked to three different-levels of the assay range in diluted samples was evaluated

Sample	Average Recovery	Range
Hu serum	87%	80%-94%
Hu EDTA Plasma	97%	95% -99%
Hu Urine	91%	87%-94%
Culture Media	87%	85%-93%

2. **Linearity**
To assess the linearity of the assay, human CRP spiked samples were diluted to produce samples with values within the dynamic range of the assay.

		Cell culture media	EDTA-plasma	Serum	Urine
1:2	%Expected	103	97	100	99
1:4	%Expected	101	108	101	97
1:8	%Expected	110	113	99	99

3. **Sensitivity:** 35pg/mL

4. **Precision**
Human serum, plasma and culture media samples with different levels of CRP were assayed 10 times each on

three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Intra-assay:

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
Serum (n=10)	4.50	4.14	6.19	4.94
Heparin-Plasma (n=10)	6.57	3.82	4.29	4.89
Culture Media (n=10)	6.16	4.47	7.97	6.20

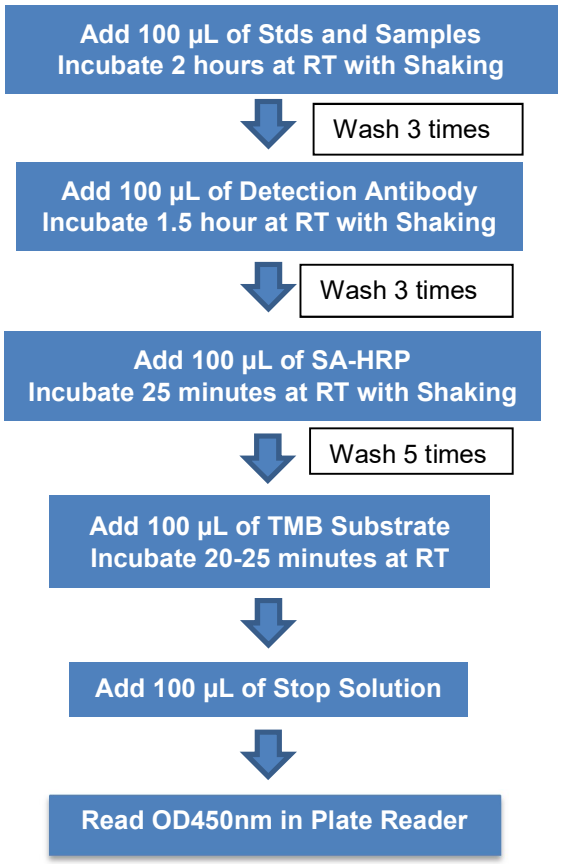
Inter-assay

Sample	Mean (ng/ml) in assay1	Mean (ng/ml) in assay2	Mean (ng/ml) in assay3	Ave (pg/ml)	SD	%CV
Serum (n=10)	0.66	0.64	0.62	0.64	0.02	3.04
EDTA-Plasma (n=10)	1.10	1.13	1.10	1.11	0.02	1.37
Culture Media (n=10)	0.30	0.32	0.26	0.29	0.03	10.55

Limitations of Use

1. This kit is for research use only. **Not for use in diagnostic procedures.**
2. The CRP value measured using OriGene CRP ELISA kit may not be interchangeable with that obtained from other assay kits.
3. The assay cannot be used to quantitate samples with CRP values higher than the highest standard without further dilution of the samples.

Assay Flowchart



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