Quantikine® QuicKit™ ELISA

Human C-Reactive Protein/CRP Immunoassay

Catalog Number QK1707

For the quantitative determination of human C-Reactive Protein (CRP) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

C-Reactive Protein (CRP), also known as Pentraxin 1, is a non-glycosylated protein in the Pentraxin family that also includes Pentraxin 2/SAP and Pentraxin 3/TSG-14. CRP functions as a sensor and activator of the innate immune response (1). In humans, it is a major acute-phase protein; its circulating concentration is dramatically elevated at the onset of inflammation (2). In mice, however, serum CRP levels increase only slightly during inflammation, and the analogous acute phase role is filled by Pentraxin 2 (3). CRP assembles non-covalently into a 110-120 kDa cyclical pentamer (4). Mature human CRP shares 71% and 64% amino acid (aa) sequence identity with mouse and rat CRP, respectively (5).

CRP binds and opsonizes apoptotic cells (6-8) as well as bacteria such as S. *pneumoniae* (9, 10). It subsequently enhances the phagocytosis of these opsonized cells (6, 8-10). CRP additionally binds several proteins in the complement cascade including C1q, C4BP, and Factor H (8, 11-13). It enhances activation of the classical complement pathway and the deposition of C3b (9). In later stages of the response, CRP inhibits complement-mediated cell lysis through its binding to C4BP and Factor H (8, 12). These interactions induce the upregulation of complement inhibitory proteins CD46, CD59, and CD55/DAF and inhibit assembly of the membrane attack complex (MAC) (8, 14).

CRP binds to Fcγ RI, Fcγ RIIA, and Fcγ RIIB on macrophages and dendritic cells (15-17), and Fc receptors are required for the phagocytosis of CRP-opsonized target cells (6, 10, 18). CRP binding to Fcγ RI induces Src activation which subsequently triggers the inhibitory Fcγ RIIb and dampens the inflammatory response (15, 19). CRP additionally promotes dendritic cell maturation and humoral immunity (10). In cardiovascular disease, CRP binds to oxidized LDL, exacerbates tissue damage in coronary artery infarction, and inhibits the repair of injured vascular endothelium (7, 19, 20).

The Quantikine® QuicKit™ Human C-Reactive Protein/CRP Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human CRP levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human CRP and antibodies raised against the recombinant protein. Results obtained using natural human CRP showed linear curves that were parallel to the standard curves obtained using the QuicKit™ standards. These results indicate that this kit can be used to determine relative mass values for natural human CRP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human CRP. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of CRP bound. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® QuicKit™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human CRP Standard	899072	2 vials of recombinant human CRP in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human CRP Capture Ab Concentrate	899070	Lyophilized tagged monoclonal antibody specific for human CRP.	May be stored for up to 1 month at 2-8 °C.*
Human CRP Detection Ab Concentrate	899071	400 µL of a monoclonal antibody specific for human CRP conjugated to horseradish peroxidase with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Assay Diluent RD1-36	895272	11 mL of a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human CRP Controls (optional; R&D Systems®, Catalog # QC265)

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 100-fold dilution due to high endogenous levels. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Assay Diluent RD1-36 (diluted 1:2) - Add 5.0 mL of Assay Diluent RD1-36 to 5.0 mL of deionized or distilled water to prepare 10 mL of Assay Diluent RD1-36 (diluted 1:2).

Human CRP Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CRP Capture Ab Concentrate with Assay Diluent RD1-36 (diluted 1:2). This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-36 (diluted 1:2). For a full plate, add 300 µL of reconstituted Human CRP Capture Ab stock and 300 µL of Human CRP Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-36 (diluted 1:2).

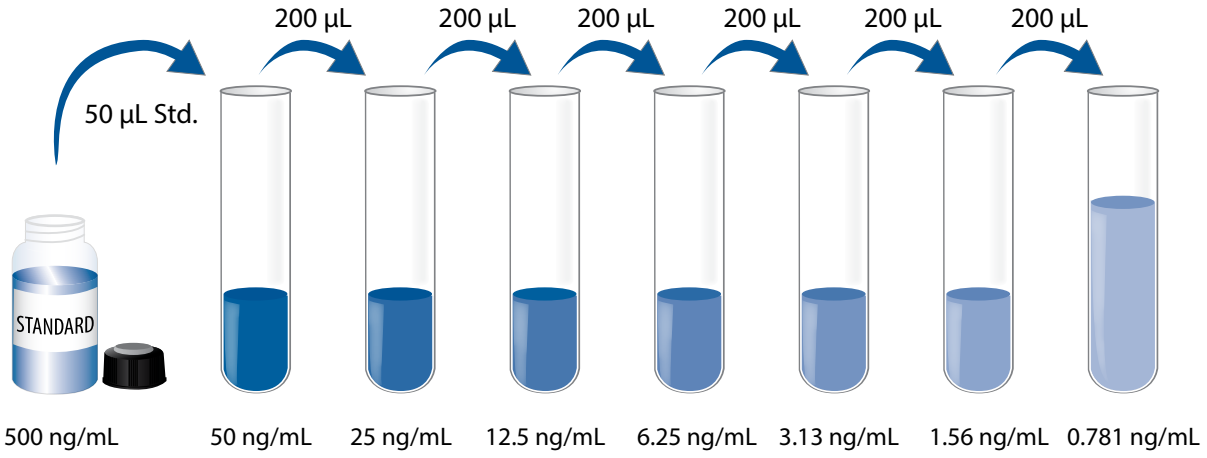
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human CRP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CRP Standard with deionized or distilled water. This reconstitution produces a stock solution of 500 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 50 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of standard, control, or sample* per well.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 50 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.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

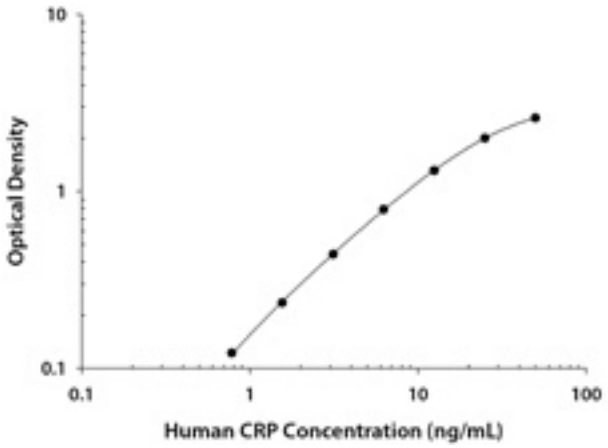
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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. The data may be linearized by plotting the log of the human CRP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.004 0.005	0.005	——
0.781	0.125 0.129	0.127	0.122
1.56	0.238 0.241	0.240	0.235
3.13	0.441 0.450	0.446	0.441
6.25	0.778 0.813	0.796	0.791
12.5	1.319 1.337	1.328	1.323
25	1.995 2.015	2.005	2.000
50	2.594 2.634	2.614	2.609

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (ng/mL)	3.75	26.1	4.03	24.8
Standard deviation	0.146	1.08	0.195	1.31
CV (%)	3.9	4.1	4.8	5.3

RECOVERY

The recovery of human CRP spiked to three levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	92-102%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CRP were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=2)	EDTA plasma* (n=2)	Heparin plasma* (n=2)
1:2	Average % of Expected	100	94	95	95
	Range (%)	95-103	91-97	93-97	95-95
1:4	Average % of Expected	98	97	99	98
	Range (%)	95-102	94-100	96-103	98-99
1:8	Average % of Expected	98	100	101	101
	Range (%)	93-104	95-104	97-106	99-102
1:16	Average % of Expected	98	97	103	104
	Range (%)	93-103	93-101	99-107	100-107

*Samples were diluted prior to assay.

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of human CRP ranged from 0.004-0.079 ng/mL. The mean MDD was 0.015 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human CRP produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CRP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	2546	176-9946	2835
EDTA plasma (n=20)	2443	178-9519	2719
Heparin plasma (n=20)	2401	171-9550	2627

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs) (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left unstimulated or stimulated with 10 µg/mL PHA for 5 days. Aliquots of the culture supernates were removed and assayed for levels of human CRP. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human CRP.

The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a low level recombinant human CRP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Ficolin-1
Neuronal Pentraxin 1
Neuronal Pentraxin 2
Neuronal Pentraxin R
Pentraxin 2/SAP
Pentraxin 3/TSG-14

Recombinant mouse:

CRP
Pentraxin 2/SAP
Pentraxin 3/TSG-14

Recombinant rat:

CRP
Pentraxin 2/SAP

Other recombinant:

porcine CRP

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