

Quantikine[®] ELISA

Human C-Reactive Protein/CRP Immunoassay

Catalog Number DCRP00

SCRP00

PDCRP00

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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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.

19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.

24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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 RIIIB 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® Human C-Reactive Protein/CRP Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CRP in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human CRP and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CRP showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit 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. A monoclonal antibody specific for human CRP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CRP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human CRP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CRP bound in the initial step. 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 with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with 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® 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps 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.

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 MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DCRP00	CATALOG # SCRPO0	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human CRP Microplate	893167	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human CRP.	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 Conjugate	893168	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human CRP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human CRP Standard	893169	1 vial	6 vials	50 ng/vial of recombinant human CRP in a buffered protein base with preservatives.	
Assay Diluent RD1F	895041	2 vials	12 vials	6 mL/vial of a buffered protein base with preservatives. <i>May contain a precipitate. Mix well before and during use.</i>	
Calibrator Diluent RD5P	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. <i>Use diluted 1:5 in this assay.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. <i>May turn yellow over time.</i>	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DCRP00 contains sufficient materials to run an ELISA on one 96 well plate.

SCRPO0 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDCRP00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- 100 mL 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human CRP Controls (Optional; R&D Systems®, Catalog # QC70).

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

Use polypropylene test tubes.

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 μ L of sample + 990 μ L of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

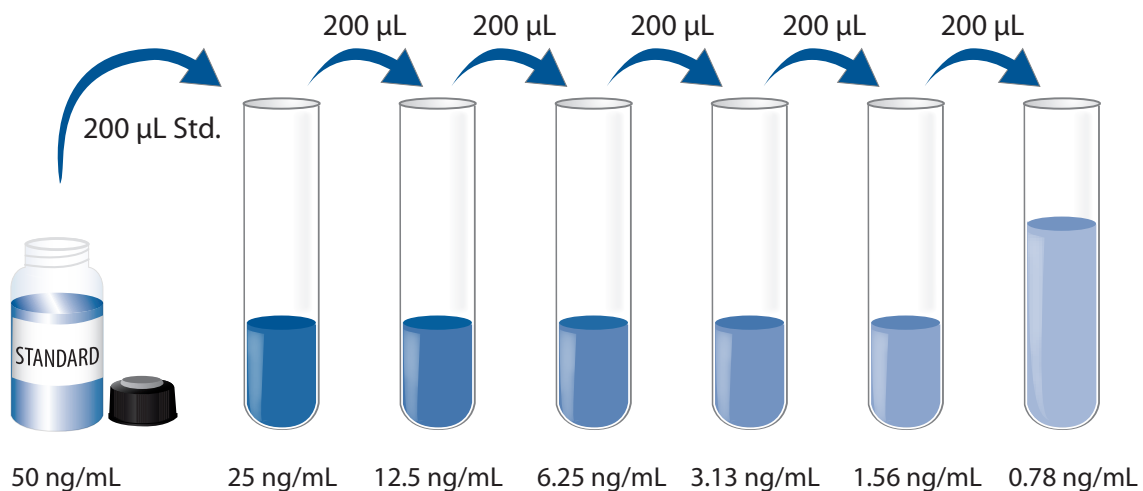
Bring all reagents to room temperature before use.

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 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. 200 μ L of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human CRP Standard - Use polypropylene tubes. Pipette 200 μ L of Calibrator Diluent RD5P (diluted 1:5) into each tube. Add 200 μ L of the standard to the 25 ng/mL tube and continue the dilution series (below). Mix each tube thoroughly before the next transfer. The Human CRP Standard (50 ng/mL) serves as the high standard. The 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples 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 100 μL of Assay Diluent RD1F to each well. *May contain a precipitate. Mix well before and during use.*
4. Add 50 μL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 μL of Human CRP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. 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.
10. 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

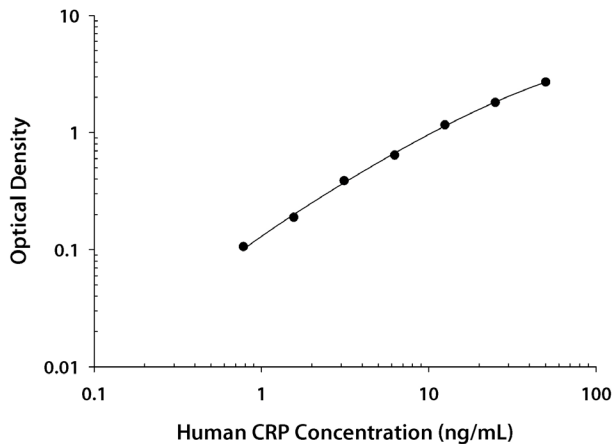
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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	0.009 0.010	0.010	—
0.78	0.112 0.119	0.116	0.106
1.56	0.194 0.204	0.199	0.189
3.13	0.392 0.406	0.399	0.389
6.25	0.645 0.656	0.651	0.641
12.5	1.167 1.176	1.172	1.162
25	1.805 1.834	1.820	1.810
50	2.676 2.746	2.711	2.701

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	4.79	8.66	18.9	4.84	8.66	17.5
Standard deviation	0.21	0.33	1.57	0.29	0.61	1.15
CV (%)	4.4	3.8	8.3	6.0	7.0	6.6

RECOVERY

The recovery of human CRP spiked to levels throughout the range of the assay in cell culture media was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	92-110%

LINEARITY

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

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	107	101	99	102
	Range (%)	98-112	89-105	95-100	93-111
1:4	Average % of Expected	102	100	96	98
	Range (%)	91-109	84-111	89-106	92-104
1:8	Average % of Expected	100	97	94	96
	Range (%)	88-108	84-108	89-99	91-101
1:16	Average % of Expected	95	97	89	94
	Range (%)	86-104	86-102	88-91	91-97

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human CRP ranged from 0.005-0.022 ng/mL. The mean MDD was 0.010 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®.

The recombinant protein is directly calibrated to the NIBSC/WHO First International Standard 85/506.

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=35)	1769	109-4291	1214
EDTA plasma (n=35)	1547	104-4185	1082
Heparin plasma (n=35)	1624	108-4523	1138

Note: *Four additional serum, EDTA plasma, and heparin plasma patient sample sets were tested and resulted in substantially higher values. The values for each donor averaged 7364 ng/mL, 12,363 ng/mL, 16,064 ng/mL, and 43,036 ng/mL.*

Cell Culture Supernates - Human peripheral blood cells (1×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernate 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 mid-range recombinant human CRP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Recombinant rat:
Pentraxin 2/SAP	CRP	CRP
Pentraxin 3/TSG-14	Pentraxin 3/TSG-14	Pentraxin 2/SAP

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