C-Reactive Protein (human) ELISA Kit

Item No. 10011236

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400310	Anti-CRP (human) Coated Plate	1 plate
400311	Anti-CRP (human) HRP Conjugate (100X)	1 vial/0.13 ml
400312	CRP (human) ELISA Standard	1 vial/0.13 ml
400313	CRP (human) Assay Buffer Packet	1 packet
400314	CRP TMB Substrate	1 vial/12 ml
400315	CRP HRP Stop Solution	1 vial/12 ml
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
E-Mail:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box. Bring all reagents to room temperature 30 minutes before starting.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of 'UltraPure' water. Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 8).

INTRODUCTION

Background

C-Reactive Protein (CRP) is a 224 amino acid protein that is synthesized primarily by hepatocytes, and to a lesser extent adipocytes. CRP plasma levels increase ~1,000-fold in response to acute and chronic inflammatory conditions, making it a useful gauge of inflammation in a wide range of physiological and pathological conditions.[1] Normal levels of serum CRP (0.64 µg/ml) do not differ between healthy adult men and women, but tend to increase slightly with age.[2] High plasma CRP concentrations (>3 µg/ml) are associated with an increased risk for atherosclerosis.[3] CRP has been implicated as a contributor to atherogenesis by modulating endothelial function, stimulating coagulation, inducing the expression of ICAM-1, VCAM-1, and E-selectin, mediating uptake of low-density lipoproteins into macrophages, and destabilizing plaques.[1,4-9] In addition, CRP can bind in a calcium-dependent manner to phosphocholine on microbes and act as a ligand for specific receptors on phagocytic leukocytes. CRP mediates the activation of monocytes and macrophages *via* IL-6, TNF-α, and other cytokines, and assists in the complement pathway.[1,10-12]

About This Assay

Cayman's CRP (human) ELISA Kit is an immunometric assay which can be used to measure CRP in plasma without prior sample purification. The assay has a range from 46.9-3,000 pg/ml and a limit of detection of approximately 46.9 pg/ml.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for human CRP (mouse anti-human CRP). This antibody will bind any human CRP introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of an HRP-labeled CRP monoclonal antibody to detect the captured CRP. The two antibodies form a 'sandwich' by binding to different locations on the CRP molecule. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound HRP-labeled monoclonal antibody, which in turn is proportional to the concentration of the CRP.

$$\text{Absorbance} \propto [\text{Anti-CRP HRP}] \propto [\text{CRP}]$$

A schematic of this process is shown in Figure 1, on page 7.

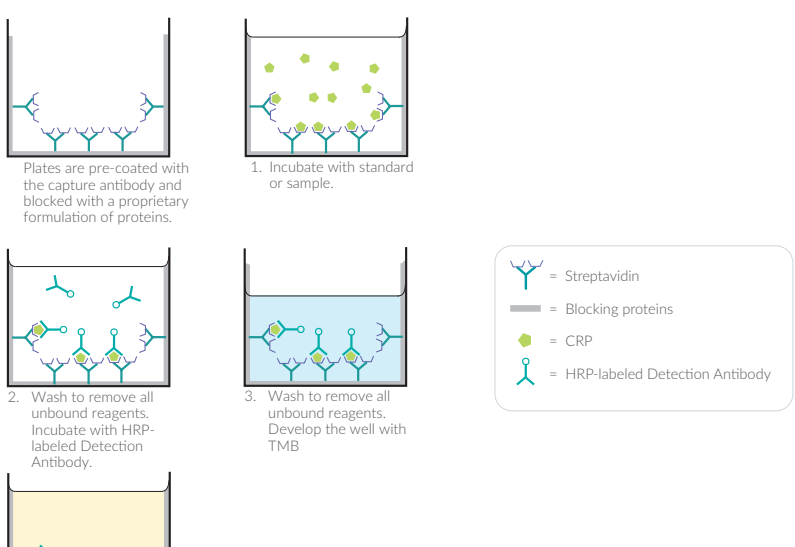

Figure 1. Schematic of the AChE ELISA

Definition of Key Terms

Blank: background absorbance caused by TMB. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

CRP (human) Assay Buffer Preparation

Reconstitute the contents of the CRP (human) Assay Buffer Packet (Item No. 400313) with 1 L of UltraPure water. The reconstituted Assay Buffer will be stable for approximately two months when stored at 4°C.

Sample Preparation

In general, human plasma (prepared using heparin or EDTA as the anticoagulant) can be used directly in the assay following dilution, as described below.

NOTE: Standards and samples should be freshly diluted before running this assay. Avoid freezing and thawing samples multiple times. Plasma samples should be stored at -80°C.

Plasma

To prepare plasma samples for use in the ELISA, centrifuge them at maximum speed for five minutes at room temperature using a benchtop centrifuge. Carefully transfer the samples to clean tubes avoiding any lipid or cell debris in the tubes. Samples obtained from normal healthy individuals will generally fall on the standard curve of the assay when diluted between 1:1,000 and 1:16,000. We recommend diluting one or two typical samples at multiple dilutions within this range and measuring their CRP content by ELISA. Once you have determined the appropriate dilution range for your typical samples, the remainder of your samples can be diluted in a similar manner for use in the immunoassay.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Anti-CRP (human) HRP Conjugate (100X)

Dilute the vial of Anti-CRP (human) HRP Conjugate (100X) (Item No. 400311) with 13 ml Assay Buffer. The diluted conjugate should be used within 24 hours. *NOTE: Protect diluted conjugate from light.*

CRP (human) ELISA Standard

Equilibrate a pipette tip in Assay Buffer by repeatly filling and expelling the tip with Assay Buffer several times. Using the equilibrated pipette tip, transfer 10 µl of the CRP (human) ELISA Standard (Item No. 400312) into a clean test tube, dilute with 990 µl Assay Buffer and mix gently. The concentration of this solution (the bulk standard) will be 10 ng/ml.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them, #1 through #8. Aliquot 700 µl of Assay Buffer to tube #1, and 500 µl of Assay Buffer to tubes #2-8. Transfer 300 µl of the bulk standard (10 ng/ml) to tube #1 and mix gently. Serially dilute the standard by removing 500 µl from tube #1 and placing into tube #2. Mix gently. Next, remove 500 µl from tube #2 and place into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any CRP Standard to tube #8. This tube is the zero-point vial, the lowest point on the standard curve.

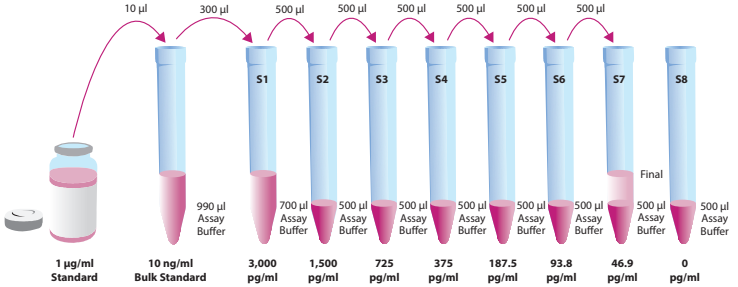

Figure 2. Preparation of the CRP standards

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, plate the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 13 for more details). We suggest you record the contents of each well on the template sheet provided (see page 18).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	Blk
H	S8	S8	8	8	8	16	16	16	24	24	24	Blk

Blk - Blank
S1-S8 - Standards 1-8
1-26 - Samples

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. **CRP (human) ELISA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all of the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

2. **Samples**

   Add 100 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

3. **Incubate the Plate**

   Cover each plate with plastic film (Item No. 400012) and incubate for one hour at room temperature on an orbital shaker.

4. **Wash**

   Empty the wells and rinse four times with Assay Buffer.

5. **Anti-CRP (human) HRP Conjugate**

   Add 100 µl to each well except the Blk wells.

6. **Incubate the Plate**

   Cover each plate with plastic film (Item No. 400012) and incubate for 30 minutes at room temperature on an orbital shaker.

Development of the Plate

1. **Wash**

   Empty the wells and rinse four times diluted Assay Buffer. Each well should be completely filled with Assay Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbant paper to remove the residual Assay Buffer.

2. **CRP TMB Substrate Solution**

   Add 100 µl of CRP TMB Substrate Solution (Item No. 400314) to each well.

3. **Incubate the Plate**

   Cover the plate with plastic film and incubate for 15 minutes at room temperature in the dark. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark.

4. **CRP HRP Stop Solution**

   DO NOT WASH THE PLATE. Add 100 µl of CRP HRP Stop Solution (Item No. 400315) to each well. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Read the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. We recommend using a second order polynomial (quadratic) fit since the absorbance at the standard concentrations is slightly non-linear. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance values from each of the standard wells.
2. Average the absorbance values for each unknown sample.

Plot the Standard Curve

Plot absorbance for standards S1-S8 (y-axis) *versus* CRP (human) concentration (x-axis) using linear axes and fit the data with a quadratic equation.

Determine the Sample Concentration

1. Use a calculator or spreadsheet to determine the equation for the quadratic regression curve.
2. Use this equation to calculate the value of your samples.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Dose (pg/ml)	Absorbance		Average
3,000	2.712	2.583	2.648
1,500	1.544	1.555	1.550
750	0.861	0.820	0.841
375	0.490	0.442	0.466
187.5	0.230	0.235	0.233
93.8	0.123	0.114	0.119
46.9	0.067	0.069	0.068
0	0.008	0.048	0.028

Table 1. Typical results

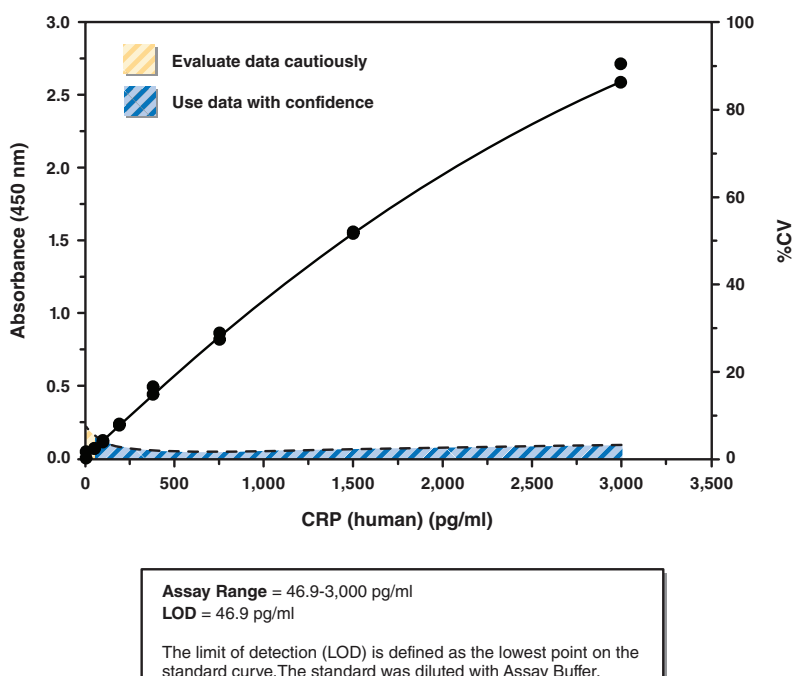

Figure 4. Typical standard curve

Precision:

The intra-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 15 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation
3,000	3.0
1,500	2.6
750	1.9
375	2.9
187.5	2.2
93.8	2.0
46.9	7.0
0	†

Table 2. Intra-assay variation

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

†Outside of the recommended usable range of the assay.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
Poor development (low signal) of standard curve	A. Standard was diluted incorrectly B. Standard is degraded	A. Check dilution protocol and re-assay

References

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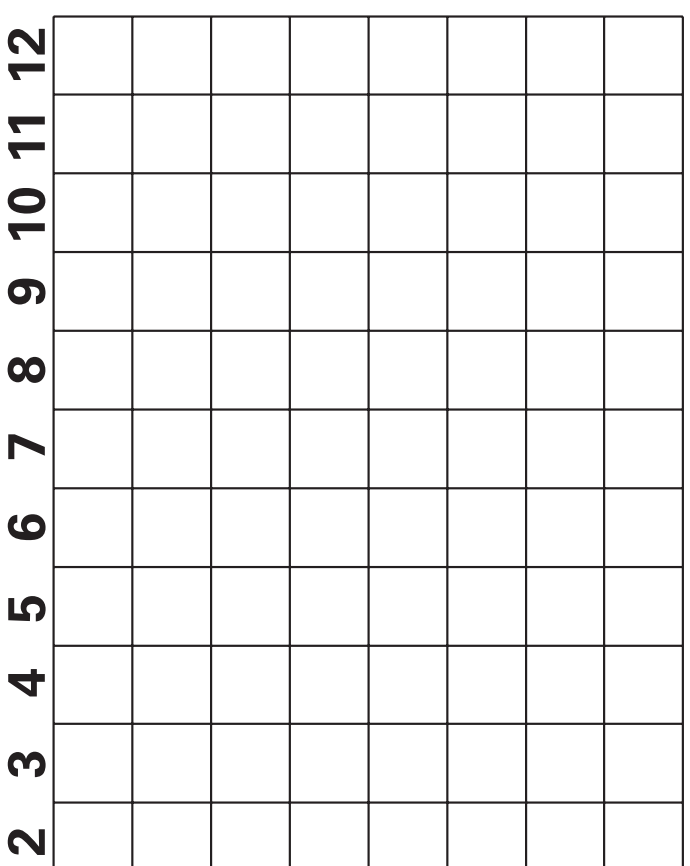

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.