REFERENCES

1. Thompson D, Pepys MB, Wood SP (1999). Structure 7 (2): 169–77
2. Pepys MB, Hirschfield GM (June 2003). J. Clin. Invest. 111 (12): 1805–12.
3. Lau DC, Dhillon B, Yan H, et al. (May 2005). Am. J. Physiol. Heart Circ. Physiol. 288 (5): H2031–41.
4. Mantovani A, Garlanda C, Doni A, Bottazzi B (January 2008). J. Clin. Immunol. 28 (1): 1–13.
5. Clearfield MB (2005). The Journal of the American Osteopathic Association 105 (9): 409–16.

Chicken CRP Immunoassay

Catalog Number: SEKCN-0100

For the quantitative determination of chicken CRP concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd.
Address:NO.85, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 400-968-6088 Fax: 86-10-56371282 E-mail: service@solarbio.com

TABLE OF CONTENTS

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

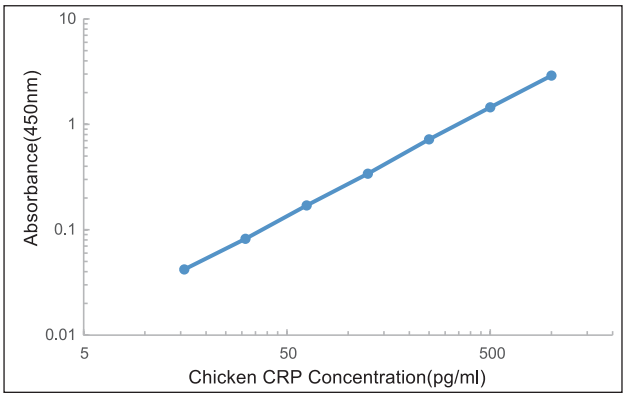
RECOVERY: The recovery of CRP spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CRP in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	92	84-101
Cell culture supernatants	95	98-106

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CRP in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	97	105
	Range (%)	89-106	96-115
1:4	Average% of Expected	94	108
	Range (%)	86-102	99-117



Representative standard curve for CRP ELISA.

PERFORMANCE CHARACTERISTICS

SENSITIVITY: The minimum detectable dose was 6 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant Chicken CRP. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

BMP1, BMP2, BMP4, HGF, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFN, MMP-2, sIL-2R, sIL-6R, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF

BACKGROUND

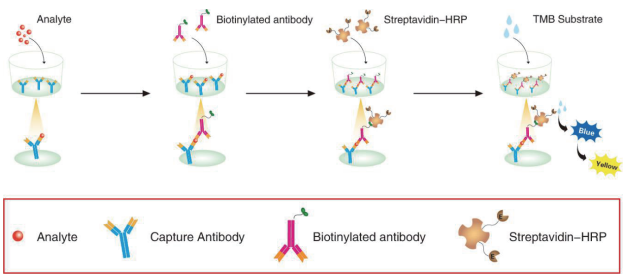
C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (i.e. CRP is an acute-phase protein). CRP was first identified as a substance in the serum of patients with acute inflammation that reacted with the C-polysaccharide of Pneumococcus. Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins.C-reactive protein was the first pattern recognition receptor (PRR) to be identified. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause). CRP is used mainly as a marker of inflammation and infection. Measuring CRP level is a screen for infectious and inflammatory diseases. Rapid, marked increases in CRP occur with inflammation, infection, trauma and tissue necrosis, malignancies, and autoimmune disorders. Because there are a large number of disparate conditions that can increase CRP production, an elevated CRP level does not diagnose a specific disease. An elevated CRP level can provide support for the presence of an inflammatory disease, such as rheumatoid arthritis, polymyalgia rheumatica or giant-cell arteritis. However, CRP level is an independent risk factor for atherosclerotic disease. Patients with high CRP concentrations are more likely to develop stroke, myocardial infarction, and severe peripheral vascular disease.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CRP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CRP present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for CRP is added to detect the captured CRP protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by

tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CRP ELISA

Std (pg/mL)	O.D.1	O.D.2	Average	Correct
0	0.038	0.036	0.037	---
15.625	0.115	0.117	0.116	0.079
31.25	0.178	0.182	0.180	0.143
62.5	0.336	0.348	0.342	0.305
125	0.618	0.635	0.626	0.589
250	1.143	1.164	1.153	1.116
500	1.775	1.751	1.763	1.726
1000	2.833	2.856	2.844	2.807

used. make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

5.**Working solution of Streptavidin-HRP(120μL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120μL HRP Conjugate sufficient for 96-well plate.Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100μLof HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-Chicken CRP antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature (25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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℃**
Standard-lyophilized,2000pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C **for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C **for six months
Substrate Solution	1 bottle	Store at 2-8°C **for six months
Stop Solution	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. 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 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

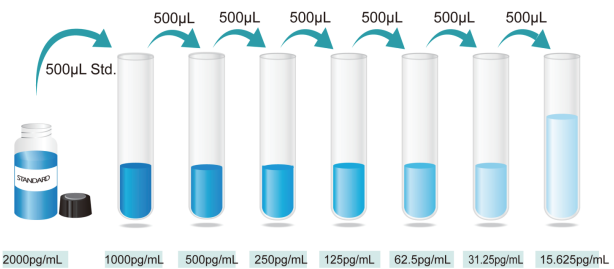
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample (2 vials) –** Chicken CRP Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of **Standard /sample**

Diluent. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette 500µL of Standard/Sample Diluent into 1000pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Chicken CRP standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Chicken CRP antibody(1 vials)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is