ELISA kits available from ADI:

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha
Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin

T**urkey**: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

See Details at the web site or Contact ADI

Instruction Manual No. M-1040

Mouse C-Reactive Protein (CRP)

ELISA KIT Cat. No. 1040

For Quantitative Determination of CRP In Mouse Serum

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Mouse CRP ELISA KIT Cat. No. 1040

Kit Components, 96 tests	Cat #
Anti-Mouse CRP coated strip plate (8 wells x 12 strips)	1041
Mouse CRP Reference Standard, lyophilized Reconstitute with 0.200 ml distilled water	1042
Anti-Mouse CRP-HRP Conjugate, 11 ml	CRP-HRP
1x Sample Diluent, 30 ml	CRP-SD
20x Wash Buffer, 50 ml	WB-20
TMB Substrate, 11 ml	TMB-CRP
Stop solution, 11 ml	CRP-S
Instruction Manual	M-1040

INTRODUCTION

C-reactive protein (CRP) has been regarded as an acute phase reactant in serum (1). It consists of five single subunits, which noncovalently linked and assembled, as a cyclic pentamer with a mol. Wt. Range of 110-140 kDa (2). CRP has been found to be increased in serum of patients with a wide variety of diseases including infections by gram-positive and gram-negative bacteria (3), acute phase of rheumatoid arthritis (4), abdominal abscesses, inflammation of bile ducts (4), myocardial infarction (4, 5), and malignant tumors (6, 7). CRP may be found in patients with Guillain-Barre syndrome and multiple sclerosis (8), certain viral infections (6, 9), tuberculosis (4, 7), acute infectious hepatitis (6), many other necrotic and inflammatory diseases, burned patients, and after surgical trauma (4). Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is useful indicator of inflammatory processes. CRP levels rise in serum within hours of the onset of inflammation, reach a peak during the acute stage and decrease with resolution of inflammation trauma. The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate, which may also be influenced by physiological changes not associated with an inflammation process. Current quantification methods including latex agglutination, nephelometry, and radial immunodiffusion have the general disadvantage accompany agglutination and precipitation techniques (10).

ADI's Mouse CRP ELISA provides is a very specific and sensitive assay for Mouse CRP. This kit is designed to measure CRP levels in Mouse serum. Page 1 Rev. 1040/70417G

PERFORMANCE CHARACTERISTICS

Detection Limit: Based on 6 replicate determinations of the zero standards, the minimum CRP concentration detectable using this assay is ~2.0 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: A limited testing of 20 adult mouse serum samples produced values of 4 -14 ug/ml (average 6.5 ug/ml).

Species Crossreactivity
Cross reactivity was tested with the following CRP proteins at 100 ng/ml: Rat CRP showed >200% reactivity, and human CRP showed ~10% reactivity. Monkey, rabbit and dog CRP all showed less than 1% cross reactivity. The following sera showed less than 1% reactivity when diluted at 1:100: bovine, FBS, hamster, guinea pig, rat, sheep, chicken, pig and goat.

ADI provides CRP ELISA kits For Human, Mouse, Rat, Monkey, Rabbit and Dog.

References: 1. Powell L et al (1979) Am J. Med. Technol. 87, 138, 2. Osmand, AP (1977) PNAS 74, 739, 3. Ash R et al (1983) J. Infec. Immunity 53, 89; 4. Hedlulnd et al (1947) Acta Med. Scan. 128, 579; 5. Kushner I et la (1978) J Clin. Invest. 61, 235; 6. Hedlund, P et al (1961) Acta Med. Scan. 169, 1; 7. Yocum S et al (1957) Arch. Intern. Med. 99, 74; 8. Dowling P (1972) in Multiple Sclerosis, AP, pp269; 9, Roantree RJ et al (1955) Arch Int. Med. 96, 674; 10. Morley JJ et al (1982) Ann NY Acad Sci 389, 406; 11. Claus DR (1976) J Lab. Clin. Med. 87, 120.

Published Citations of ADI's Human CRP ELISA kit-

Labarrere C et al 2002 C-reactive protein, arterial endothelial activation, and development of transplant coronary artery disease: a prospective study Lancet 3600, 1462-1467

Prio TK et al 2002 Asymptomatic bacteriuria in elderly humans is associated with increased levels of circulating TNF receptors and elevated numbers of neutrophils Expt. Gero

Chen NX et al 2002 Phosphorus and uremic serum up-regulate osteopontin expression in vascular smooth muscle cells Kidney International 62, Issue 5, Page 1724

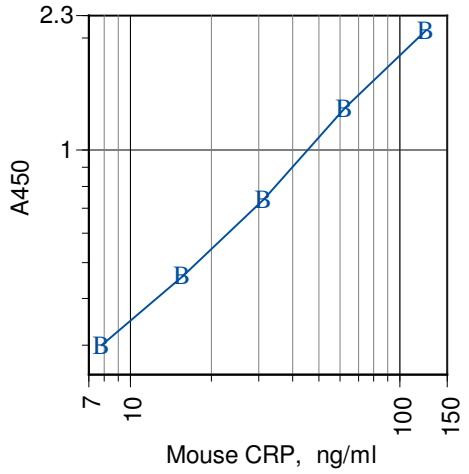
Raio L et al 2003 Evidence of fetal C-reactive protein urinary excretion in early gestation Obstetrics & Gynecology 101, 1062-1063

Bruunsgaard H et al 2003 Long-Term Combined Supplementations with -Tocopherol and Vitamin C Have No Detectable Anti-Inflammatory Effects in Healthy Men J. Nutr., Apr 2003; 133: 1170 – 1173

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Negative Diluent Control**	0.20	
B1, B2	**Standard A** 7.8 ng/ml	0.30	
C1, C2	**Standard B** 15.6 ng/ml	0.46	
D1, D2	**Standard C** 31.2 ng/ml	0.74	
E1, E2	**Standard D** 62.5 ng/ml	1.30	
F1, F2	**Standard E** 125 ng/ml	2.10	
G1, G2	**Sample 1** 1:500 dilution	1.51	80 ng/ml = **4.0** ug/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Standard Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Mouse CRP ELISA kit is based on binding of Mouse CRP from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of CRP present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of CRP in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Mouse CRP ELISA Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed , store frozen for up to six months. Avoid repeated freezing and thawing of samples. It is also possible to use plasma for testing.

REAGENT PREPARATION

1. Dilute the Sample Diluent 1:10 with water (10 ml diluent in 90ml water). Dilute only the required reagent. Store diluted solution at 2-8^{o} C for 3-4 days.

2. The Wash Buffer is a 20x stock. Dilute the entire 50ml with distilled or deionized water to 1 L total volume. Store at room temperature for the entire use of the kit.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. Reconstitute lyophilized Reference Standard with 200uL of distilled water. The stock concentration will be 5240 ng/ml. Store unused Reference Standard at -20°C.

2. Prepare liquid standards using the following dilution scheme:

Mouse CRP		Diluent	Final Concn		Final Volume
Concn	Volume				
5240 ng/ml	12 uL	+ 491 uL	**E**	125 ng/ml	253 uL
E 125 ng/ml	250 uL	+ 250 uL	**D**	62.5 ng/ml	250 uL
D 62.5 ng/ml	250 uL	+ 250 uL	**C**	31.2 ng/ml	250 uL
C 31.2 ng/ml	250 uL	+ 250 uL	**B**	15.6 ng/ml	250 uL
B 15.8 ng/ml	250 uL	+ 250 uL	**A**	7.8 ng/ml	500 uL

Diluting the mouse serum samples 1:100-1:1000 (e.g., 1-10uL serum to 1mL 1x Sample Diluent) will bring most samples into the testing range. For those testing out of the range dilute accordingly.

3. Label or mark the microtiter well strips to be used on the plate.

4. Dispense 200-300 uL of wash buffer to all wells. Let stand for 5-15 minutes, then discard or aspirate the solution. The step should be done just before adding the samples; do not allow the wells to dry at any time during the assay.

5. Pipet 100 ul standards and diluted samples into appropriate wells.

Note: for ease of loading samples it is recommended that a second uncoated microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.

6. Mix gently, and incubate at room temperature for 60 minutes.

7. Wash the wells 3 times with 300 ul of 1x wash buffer.

8. Pipette 100 ul of Ab-enzyme conjugate into each well. Mix gently, and incubate for 45 minutes at room temperature.

9. Aspirate and wash the wells 5 times with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

10. Add 100 ul of TMB Substrate into each well. Mix gently. Cover the plate and incubate for 20 minutes at room temperature. Blue color develops.

11. Stop the reaction by adding 100 ul of stop solution to all wells. Mix gently. Blue color turns yellow.

12. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 minutes after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than 125 ng/ml CRP should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor. It is possible to use normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate CRP concentrations. Read off the CRP concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:500 then the values must be multiplied by 500 and results are expressed as mg/ml.