

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

Turkey: 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

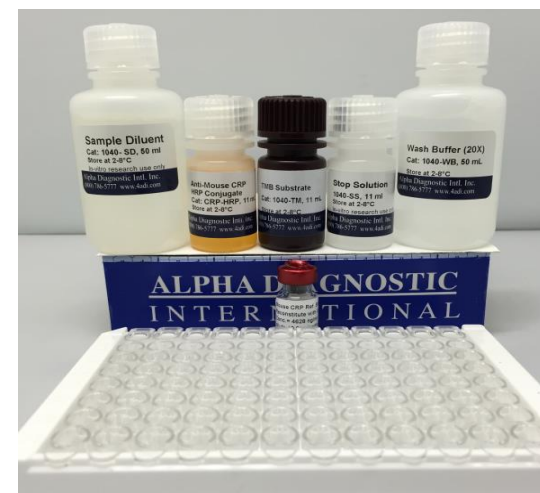
See Details at the web site or Contact ADI

Instruction Manual No. M-1040

Mouse C-Reactive Protein (CRP)

ELISA KIT Cat. No. 1040

For Quantitative Determination of CRP In Mouse Serum



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LIFE TECH
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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	1043
Sample Diluent, 50 ml	1040-SD
Wash Buffer (20X), 50 ml	1040-WB
TMB Substrate, 11 ml	1040-TM
Stop solution, 11 ml	1040-SS
Instruction Manual	M-1040

Intended Use

ADI mouse CRP ELISA is kit intended to measure CRP concentration in s mouse serum, plasma or other biological fluids. This kit is for in vitro research use only (RUO).

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.

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).

Sample Dilution

5 mouse serum (BALB/C) were 2-fold serially diluted from 1:50-:1600 and tested for CRP. Samples values were 2.44, 2.80, 6.19, and 9.87, 18.19 ug/ml with CV% 2.5-14%.

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. Infect. Immunity 53, 89; 4. Hedlund 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.

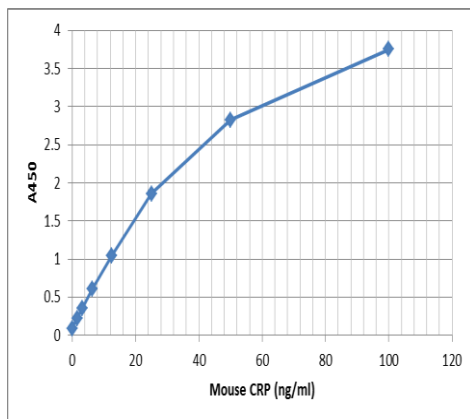
Published Citations of ADI's Human CRP ELISA kit-

Labarrere C et al	2002	Lancet 3600, 1462-1467
Prio TK et al	2002	Expt. Gerontol. 37, 693-699
Chen NX et al	2002	Kidney International 62, Issue 5, Page 1724
Raio L et al	2003	Obstetrics & Gynecology 101, 1062-1063
Brunnsgaard H et al	2003	J. Nutr., Apr 2003; 133: 1170 – 1173

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Mean A ₄₅₀ nm	Calculated Conc'n
A1, A2	0	0.090	
B1, B2	1.56	0.221	
C1, C2	3.13	0.352	
D1, D2	6.25	0.604	
E1, E2	12.50	1.047	
F1, F2	25	1.854	
G1, G2	50	2.822	
H1, H2	100	3.750	
I1, I2	Sample 1		

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.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

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. **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.
2. **Mouse CRP standard preparation:** It is supplied in powder and it is reconstituted in 0.2 ml water to give approx.. 5000 ng/ml (exact conc are lot specific). It is further diluted to prepare testing standards of 100-1.56 ng/ml in 2-fold serial dilutions (see dilution scheme on page 3).
- 3.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°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. Follow the stock concentration preparation on the vial to prepare stock mouse CRP of 100 ng/ml. **(Stock concn is lot specific and the reconstitution volume and stock concn is provided for each lot).** Store unused Reference Standard at **-20°C**.
2. Prepare other standards using the following dilution of **100 ng/ml** (prepare in step 1) stock scheme: Prepare at least 250 ul of each std to run in duplicate (100 ul/well)

Mouse CRP stock		Diluent	Final Concn	Final Volume
Concn	Volume			
H 100 ng/ml	500 uL		100 ng/ml	500 uL
G 50 ng/ml	250 uL of H	250 uL	G 50 ng/ml	250 uL
F 25 ng/ml	250 uL of G	250 uL	F 25 ng/ml	250 uL
E 12.5 ng/ml	250 uL of F	250 uL	E 12.5 ng/ml	250 uL
D 6.25 ng/ml	250 uL of E	250 uL	D 6.25 ng/ml	250 uL
C.. 3.13 ng/ml	250 uL of D	250 uL	C.. 3.13 ng/ml	250 uL
B... 1.56 ng/ml	250 uL of C	250 uL	B... 1.56 ng/ml	250 uL
A... 0 ng/ml	-	250 uL	A... 0 ng/ml	

Mouse serum samples dilution: Mouse CRP in serum is 2.5-20 ug/ml. So dilute samples 1:200 (2.00 ul serum in 398 ul diluent; it is better to take 5-10 ul sample if available for better precision). Samples that are too low or high should be tested at appropriate dilution. Do not test samples at dilution less than 1:25 to minimize background.

3. Label or mark the microtiter well strips to be used on the plate.
4. Pipet **100** ul standards and diluted samples in duplicate 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.

5. Mix gently, and **incubate on at orbital micro-plate shaker at 150 rpm at room temp.. (18-25 oC) for 45 minutes**
6. Wash the wells **5 times with 400 ul** of 1x wash buffer.
7. Pipette **100 ul of Antibody-enzyme conjugate** into each well. Mix gently, and incubate on at **orbital micro-plate shaker at 150 rpm for 45 minutes at room temperature.**

8. 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.
9. Add **100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate on **plate shaker at 150 rpm for 20 minutes** at room temperature. Blue color develops.
10. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently. Blue color turns yellow.
11. **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°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 100 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 linear 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.