ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1075

Sheep C-Reactive Protein (CRP) ELISA KIT

Cat. No. 1075, 96 tests

For Quantitative Determination of CRP
In Sheep Serum, plasma or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

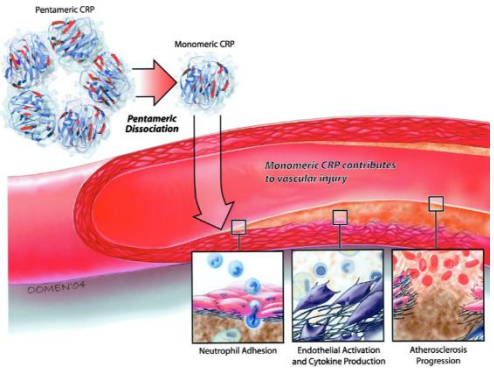
Sheep CRP ELISA KIT Cat. No. 1075

Kit Components, 96 tests	Qty #
Anti-Sheep CRP coated strip plate (8 wells x 12 strips) cat# 1076	1 plates
Sheep CRP Reference Standard, lyophilized Reconstitute with 0.2ml distilled water, cat# 1077 Note: Prepare additional standards by dilution the stock.	1 vial
Anti-Sheep CRP-HRP Conjugate, 11 ml ,cat# 1077	1 bottle
Sample Diluent, 25 ml, cat# 1077	1 bottle
20x Wash Buffer, 50 ml, cat# 1077	1 bottle
TMB Substrate, 11 ml, cat# 1077	1 bottle
Stop solution, 11 ml, cat# 1077	1 bottle
Instruction Manual, #M-1075	M-1030

Intended Use

Sheep CRP ELISA is a sandwich ELISA for the detection and measurement of CRP in sheep serum, plasma or other biological fluids. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

Introduction



C-reactive protein (CRP) has been regarded as an acute phase reactant in serum. It consists of five single subunits, which noncovalently linked and assembled, as a cyclic pentamer with a mol. Wt. Range of 110-140 kDa. 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, acute phase of rheumatoid arthritis, abdominal abscesses, inflammation of bile ducts, myocardial infarction, and malignant tumors. CRP may be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases, burned patients, and after surgical trauma. 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.

PERFORMANCE CHARACTERISTICS

Detection Limit - based on 6 replicate determinations of the zero standards, the minimum CRP concentration detectable using this assay is ~4.0 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 Sheep serum samples values of 80-120 ug/ml.

Species Crossreactivity

The antibodies used in the kit react with sheep and goat. Other species not tested.

ADI provides CRP ELISA kits For Human, Monkey, Rat, 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 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. Gerontol. 37, 693-699

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

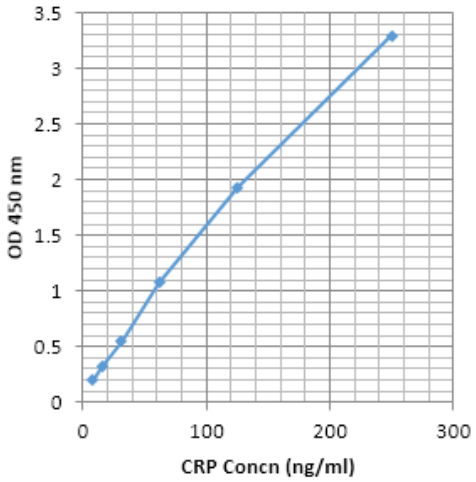
Raio L et al 2003 Evidence of fetal C-reactive protein urinary excretion in early gestation Obstetrics & Gynecology 101, 1062-1063

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	Standards & Samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	0 ng/ml Standard	0.00	
B1, B2	7.81 ng/ml Standard	0.194	
C1, C2	15.63 ng/ml Standard	0.319	
C1, C2	31.25 ng/ml Standard	0.547	
D1, D2	62 ng/ml Standard	1.073	
E1, E2	125 ng/ml Standard	1.93	
F1, F2	250 ng/ml Standard	3.29	
G1, G2	**Sample 1**	1.03	61 ng/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.

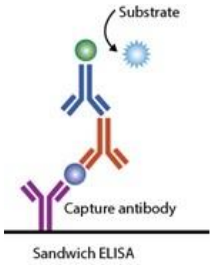


Aa/7_ADI_1075-ELISA
A typical assay Standard Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. 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 1:2000 then the values must be multiplied by 2000 and results expressed as ug/ml.

PRINCIPLE OF THE TEST



Sheep CRP ELISA kit is based on binding of Sheep 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 (blue) developed. The enzymatic reaction (color) is directly proportional to the amount of CRP present in the sample. Adding stopping solution terminates the reaction (convert blue to yellow). 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 Sheep CRP ELISA Kit is for research use only.

Standards, Sample Diluent and Antibody-HRP contain Proclin 300 (0.05%, v/v). 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 for Prolcin-300 (0.1% v/v in standards, and assay buffers).
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 , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. It is also possible to use plasma for testing.

Sample Dilution

CRP concentration in serum is 90 ug/ml in normal sheep serum. In order to obtain values within the range of the standard curve we suggest that samples be diluted 2000 fold initially, using the following procedure for each sample:

1. Dispense 98 ml and 243.75 ml of 1x diluent into two separate tubes.
2. Pipette and mix 2.0 ml of the serum/plasma sample into the first tube. This provides a 50 fold diluted sample.
3. Mix 6.25 ml of the 50 fold diluted sample with the 243.75 ml of diluent in the second tube. This provides a 2,000 fold dilution of the sample.

Reagent Preparation

Dilute the 20x Wash Buffer 1:20 with distilled or deionized water (e.g., 20ml Wash Buffer + 390ml water). Store at room temperature for 1 week.

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. Upon initial use of the kit components, remaining shelf life is 2 months with proper storage.

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

1. Reconstitute lyophilized Reference Standard with 200uL of distilled water and prepare standard as suggested on the vial (**lot sp. reconstitution volume and concn may vary and specified on the vial**). The stock concentration will be 250 ng/mL (this is only an examples). Store unused Reference Standard at -20°C.
2. Prepare additional liquid standards using2-fold serial dilution scheme:

Initial Conc.	Standard Volume (uL)	Diluent Volume (uL)	Final Volume (uL)	**Final Conc.**
250 ng/ml	-	-		**250 ng/ml**
	250 ul of 250 ng/ml	250	500	**125 ng/ml**
125 ng/ml	250 ul of 125 ng/ml	250	525	**62 ng/ml**
62 ng/ml	250 ul of 62.5 ng/ml	250	537	**31.2 ng/ml**
31.2 ng/ml	250 ul of 31.25 ng/ml	250	537	**15.63 ng/ml**
15.63 ng/ml	250 ul of 15.63 ng/ml	250	537	**7.81.2 ng/ml**

3. Dilute Sheep serum samples 1:2000 using Sample Diluent. Some samples may have to be diluted more or but 1:2000 should bring most normal samples to within the testing range. We suggest testing a few dilutions of 1-4 samples to determine appropriate dilutions of user's samples.

DILUTION OF SAMPLES

Samples containing more than 250 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.

ELISA Procedure

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **100 ul standards**, controls and diluted samples in duplicate into appropriate wells. Mix gently, cover the plate and **incubate at room temperature (25-30oC) for 45 min on an orbital shaker** at about 150 rpm (failure to shake the plate will reduce the A450.
3. **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.
4. **Wash the wells 3 times** with 250-300 ul of 1x wash buffer.
5. **Pipette 100 ul of anti-Sheep CRP-HRP conjugate** into each well. Mix gently. Cover the plate and **incubate for 45 minutes at room temperature (25-30oC) for 45 min on an orbital shaker** at about 150 rpm (failure to shake the plate will reduce the A450.
6. 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.
7. Add **100 ul of TMB** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature. Blue color develops. **Note:** TMB solution needs to be at room temperature before use.
8. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.
9. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 min 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.