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.

For In Vitro Research Use Only (RUO)

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: Techsupport@4adi.com

Web Site: www.4adi.com

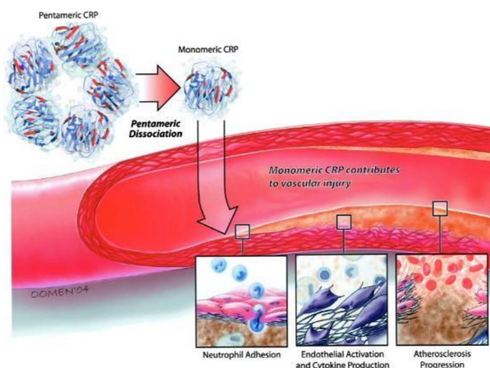
Sheep CRP ELISA KIT Cat. No. 1075

Kit Components, 96 tests	Qty #
Anti-Sheep **CRP coated strip plate** (8 wells x 12 strips) cat# 1076P	1 plate
Sheep CRP Reference Standard, **lyophilized** Reconstitute with 1.0 ml distilled water, cat# 1077 Note: Prepare additional standards by diluting the stock.	1 vial
Anti-Sheep CRP-HRP Conjugate, 11 ml ,cat# 1078	1 bottle
Sample Diluent (10X), 25 ml, cat# 1075-SD	1 bottle
Wash Buffer (20X), 50 ml, cat# 1075-WB	1 bottle
TMB Substrate, 11 ml, cat# 1075-TMB	1 bottle
Stop solution, 11 ml, cat# 1075-SS	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

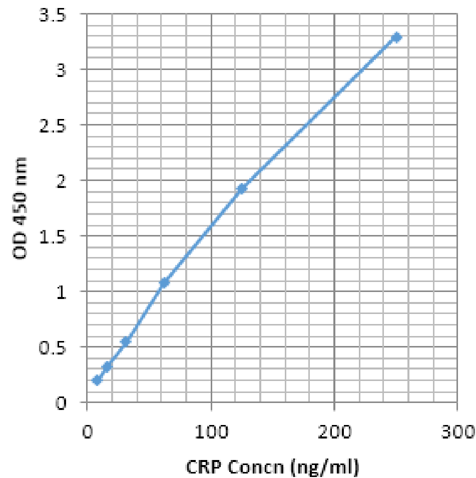
Raio L et al 2003 Evidence of fetal C-reactive protein urinary excretion in early gestation Obstetrics & Gynecology 101, 1062-1063

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	62.5 ng/ml Standard	1.073	
E1, E2	125 ng/ml Standard	1.931	
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.



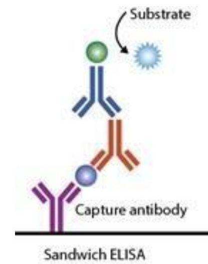
Aa/7_ADI_1075-ELISA

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.

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.
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 cannot 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 µl and 292.5 µl of 1x diluent into two separate tubes.
2. Pipette and mix 2.0 µl of the serum/plasma sample into the first tube. This provides a 50-fold diluted sample.
3. Mix 7.5 µl of the 50-fold diluted sample with the 292.5 µl 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., 50 ml Wash Buffer + 950ml water). Store at room temperature for 1 week.

Sample Diluent (10X): Dilute 1:9 with distilled or deionized water.

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 1000uL 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	500	**62.5 ng/ml**
62 ng/ml	250 ul of 62.5 ng/ml	250	500	**31.25 ng/ml**
31.2 ng/ml	250 ul of 31.25 ng/ml	250	500	**15.63 ng/ml**
15.63 ng/ml	250 ul of 15.63 ng/ml	250	500	**7.81 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).

   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.
3. **Wash the wells 5 times** with 300 ul of 1x wash buffer.
4. **Pipette 100 ul of anti-Sheep CRP-HRP conjugate** into each well. 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).
5. 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.
6. Add **100 ul of TMB** into each well. Mix gently. Cover the plate and **incubate at room temperature (25-30ºC) for 20 min on an orbital shaker** at about 150 rpm. Blue color develops. **Note:** TMB solution needs to be at room temperature before use.
7. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.
8. 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.