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

Catalog#	**ProdDescription**
9020	Pig IgG ELISA Kit, 96 tests, Quantitative (swine/porcine)
9080	Pig IgM ELISA Kit, 96 tests, Quantitative
9000	Pig Albumin ELISA Kit, 96 tests, Quantitative

See Details at the web site or Contact ADI

Instruction Manual No. M-1090

Pig C-Reactive Protein (CRP)

ELISA KIT Cat. # 1090, 96 Tests

For quantitative measurement of CRP in Pig serum or plasma

For In Vitro Research Use Only (RUO)

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

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Pig CRP ELISA KIT # 1090

Kit Components, 96 tests	#
Anti-pig CRP coated strip plate (8 wells x 12 strips), #1091P	1 plate
Pig CRP **Reference Standard, Lyophilized**, **Store at -20°C** , #1092	3 vials
HRP Conjugate, 11 ml, #1093	1 bottle
Pig CRP Sample Diluent (10X), 25 ml, #1094	1 bottle
Wash Buffer (20X), 50 ml, #1090-WB	1 bottle
TMB Substrate, 11 ml, #1090-TMB	1 bottle
Stop solution, 11 ml, #1090-SS	1 bottle
Instruction Manual, # M-1090	1

Intended Use

ADI's Pig CRP ELISA kit is a highly sensitive sandwich type assay for the quantitative measurement of CRP in pig serum or plasma. 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. 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.

ADI's Pig CRP ELISA provides is a very specific and sensitive assay for Pig CRP. This kit is designed to measure CRP levels in Pig serum or plasma.

4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of CRP in the sample.

5. Ideally, PC graphing software should be used for the above steps.

6. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

Quality Control

Full set of reference standards must be run with each run. Reference standard should closely reflect the values shown in this manual. Blanks must be less than A450=0.300. Higher blanks is an indication of poor washing. Repeat the stds only with proper washing to confirm the expected values.

PERFORMANCE CHARACTERISTICS

Wash Procedure: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

Species reactivity: The antibodies in this kit react with pig. Other species not tested. This kit is optimized for Pig CRP, Separate kits are available for detection in other species.

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

References:

1. Martinez-Subiela S, et al. A time-resolved immunofluorometric assay for porcine C-reactive protein in whole blood. Luminescence. 22:171-176 (2007)

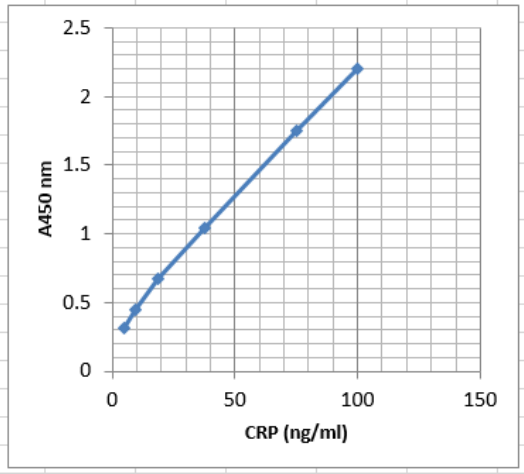
2. Pomorska-Mol M, Markowska-Daniel I and Kwik K. Immune and acute phase response in pigs experimentally infected with H1N2 swine influenza virus. FEMS Immunol Med Microbiol. 66(3):334-42 (2012)

3. Pomorska-Mol M, Kwik K and Markowska-Daniel I. Major acute phase proteins in pig serum from birth to slaughter. Bull Vet Inst Pulawy 56:553-557 (2012)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean A_{450} nm	Calculated Conc.
A1, A2	**Diluent**	0 ng/ml		
B1, B2	**Standard A**	4.67 ng/ml	0.312	
C1, C2	**Standard B**	9.38 ng/ml	0.477	
D1, D2	**Standard C**	18.75 ng/ml	0.671	
E1, E2	**Standard D**	37.5 ng/ml	1.038	
F1, F2	**Standard E**	75 ng/ml	1.615	
G1, G2	**Standard F**	150 ng/ml	2.206	

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.



*ADI_ELISA4

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

CALCULATION OF RESULTS:

1. Calculate the average absorbance values (A450) for blanks and each set of reference standards and samples. Deduct the average blank values from the stds and samples (net values).

2. Construct a standard curve by plotting the net mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP in ng/ml from the standard curve.

PRINCIPLE OF THE TEST

Pig CRP ELISA kit is based on binding of Pig 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. Plate shaker or orbital shaker; Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

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 Prolcin-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 cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

Preparation of Sample:

CRP is present in normal pig serum at concentrations of 5-30 ug/ml and levels can increase 10 fold during infection. In order to obtain values within the range of the standard curve we suggest that samples initially be diluted 500 fold using the following procedure for each sample to be tested:.

1. Dispense 998 µl of 1x diluent into a polypropylene or glass tube.

2. Pipette and mix 2 µl of the serum/plasma sample with the 998 µl of 1x diluent. This provides a 500 fold diluted sample.

3. Repeat this procedure for each sample to be tested.

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit. It can be stored at 4oC for long term storage.

2. **Sample Diluent** is 10X. **Dilute 1:10** with water (1 ml stock in 9 ml water). Store 1x sample diluent at 4oC..

3. **Reference Standard** is provided as **lyophilized powder**. Dilute it with 1X CRP sample diluent to make standards as given below.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label. The Pig CRP reference standard should be **stored at -20°C.**

Preparation of Standards

1. The concentration of the **reference lyophilized standard** (light Pink colored powder) is provided on the vial ug/ml. This is reconstituted in 1 ml of 1X Diluent to prepare stock solution and further diluted to **prepare 150 ng/ml standard** (**Std 6**) is also given on the vial. Use standards within 4-hrs of reconstitution. Prepare other refs. standards (75-4.67 ng/ml by 2-fold serial dilution) fresh prior to the assay and do not store for more than 1-2 hr. Immediately aliquot and store any unused **stock reference standard at –20°C or below**.

2. Add 1 ml of 1x diluent to one of the pig CRP standard vials and mix gently until dissolved. Use the standard within 4 hours of reconstitution and discard after use.

3. Label 6 polypropylene or glass tubes: 150, 75, 37.5, 18.75, 9.38 and 4.67 ng/ml.

4. Prepare a 150 ng/ml working CRP standard as detailed on the standard vial label, by mixing the indicated volume of diluent and reconstituted standard in the tube labeled 150 ng/ml.

4. Dispense 250 µl of diluent into the tubes labeled 75, 37.5, 18.75, 9.38 and 4.67 ng/ml.

5. Prepare a 75 ng/ml standard by diluting and mixing 250 µl of the 150 ng/ml standard with 250 µl of diluent in the tube labeled 75 ng/ml. Similarly prepare the 37.5, 18.75, 9.38 and
1. 4.67 ng/ml standards by serial dilution**.**

Notes: When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme.

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

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

1. Use first 2 wells for blanks (100 ul of 1x sample diluent). Pipet **100 ul standards and samples** in duplicate into appropriate wells. Mix gently, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (100-150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

2. Remove or aspirate the plate contents and **wash the wells 5 times** with 300 ul of 1x wash buffer using an automated washer. If washing manually dump the plate contents and tap over paper towels, add wash buffer, shake the contents 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

3. Pipette **100 ul of HRP conjugate** into each well, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (100-150 rpm).**

4. Remove or aspirate the plate contents and **wash the wells 5 times** with 300 ul of 1x wash buffer as above in step 5.

5. Add **100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature **on an orbital shaker (100-150 rpm). Blue color develops in standards and positive wells**. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 30 seconds. **Blue color turns yellow.**

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

Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and redetermined.