PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of purified Rat CRP were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 92 to 110%, indicating accurate quantitation of CRP in Rat Serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High CRP Spike		47.6	
+ Rat E, 4.0 ng/ml	51.6	52.5	**102 %**
+ Rat F, 3.5 ng/ml	51.1	56.1	**110 %**
+ Rat G, 4.9 ng/ml	52.5	57.7	**110 %**
Low CRP Spike		5.8	
+ Rat E, 4.0 ng/ml	9.8	9.0	**92 %**
+ Rat F, 3.5 ng/ml	9.3	8.7	**94 %**
+ Rat G, 4.9 ng/ml	10.7	10.7	**100 %**

Catalog# Description

1000 Human C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1010 Rat C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1020 Dog C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1030 Rabbit C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1040 Mouse C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1050 Monkey C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1055 Cow C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1060 Cat C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1065 Goat C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1070 Horse C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1075 Sheep C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1080 Rabbit C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative
1090 Pig C-Reactive Protein (CRP) ELISA Kit, 96 tests, Quantitative

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-1010

Rat Serum CRP ELISA Kit

Cat. No. 1010, 96 Tests

For Quantitative Determination of C-Reactive Protein (CRP) in Serum, plasma or other biological fluids

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

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

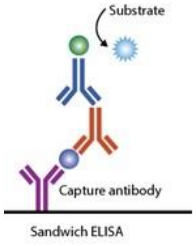
INTENDED USE

The Alpha Diagnostics Int'l Rat CRP ELISA Kit is a sandwich ELISA suitable for quantifying circulating serum CRP in rats used in the research of diseases and inflammatory conditions. The assay is configured using Rat Serum standards which produce a standard curve linear with dilutions of Rat Serum samples, and therefore provides for appropriately accurate and precise quantification of CRP in such samples.

RESEARCH USE OF THE TEST

C-reactive protein (CRP) is regarded as an acute phase reactant in serum, consisting as five non-covalently linked subunits, assembled as a cyclic pentamer, MW 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 a 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.

PRINCIPLE OF THE TEST



The Rat CRP ELISA kit is based on the binding of Rat CRP in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of CRP present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of CRP in samples and control is calculated from a curve of standards containing known concentrations of CRP.

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 box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity
The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with CRP, and have essentially no reactivity with immunoglobulins or any other rat serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, hamster, guinea pig, human, monkey, bovine, pig, horse, sheep, goat, dog, cat, rabbit, chicken and 10% neonatal bovine serum.

Normal Range
Assay values of CRP in sera from 20 adult rats ranged from 94 to 494 ug/ml (mean: 341 ug/ml). Each laboratory should determine expected values of its own testing population.

Precision
Samples containing low, medium and high concentrations of CRP, representing 3 different sera, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

CRP concentrations were measured with good within-assay (5.1 to 11.8 %CV) and very good between-assay (4.6 to 8.7 %CV) reproducibility.

Sample	CRP ng/ml	Intra-assay %CV	Inter-assay %CV
Rat A	3.6	**5.8**	**8.4**
Rat B	9.7	**5.1**	**8.7**
Rat C	39.5	**11.8**	**4.6**

Linearity of Dilution
Two individual rat sera and pooled rat sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 95 to 100%, demonstrating linear dilution and equivalent quantitation across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Rat D	1:15k 1:135k	29.7 3.2	445 432	**99 %**
Rat E	1:15k 1:135k	27.9 3.4	418 459	**95 %**
Rat Pool	1:15k 1:135k	43.2 4.85	648 655	**100%**

Continued on Page 7.

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, CRP concentrations may be determined as follows:

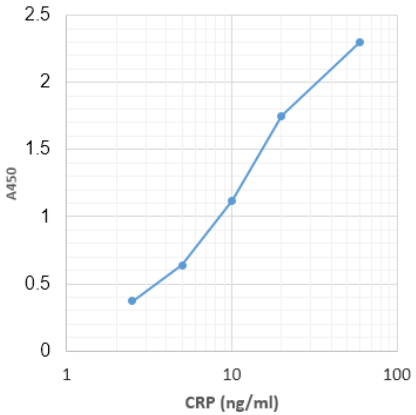
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of CRP (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The CRP concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 60 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	**CRP** ng/ml
A1, A2	**Negative Diluent Control**	0.02	0
B1, B2	2.5 ng/ml **Standard**	0.37	2.5
C1, C2	5 ng/ml **Standard**	0.64	5
D1, D2	10 ng/ml **Standard**	1.12	10
E1, E2	20 ng/ml **Standard**	1.75	20
F1, F2	60 ng/ml **Standard**	2.30	60
G1, G2	**Positive Serum Control** [Value: 5.6 – 8.4 ng/ml]	0.89	7.1
H1, H2	**Sample** [Diluted 1:45k]	1.09	9.5
	Calculated: 45k dilution x 9.5 ng/ml = **427** ug/ml in serum		

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



4-B/ELISA-1010

KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Rat CRP - HRP Conjugate Concentrate (100x) Part No. 1014, 0.15ml	Peroxidase conjugated anti-rat CRP in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8º C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Rat CRP Microwell Strip Plate	1011	8-well strips (12)	Coated with purified anti-Rat CRP antibodies.
Rat CRP Standards			
2 ng/ml 5 ng/ml 10 ng/ml 20 ng/ml 60 ng/ml	1013B 1013C 1013D 1013E 1013F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing Rat Serum with calibrated CRP concentrations; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Positive Control [CRP] range on label	1012	0.65 ml	Rat Serum with stated CRP concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Rat CRP-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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 and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

QUALITY CONTROL

Reagents

Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls

A Positive Serum Control is provided with the kit, assigned with a CRP concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run; OD should be <0.3.

Standard Curve

The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique
Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment
Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of 1:5k–1:50k are appropriate for most normal rat sera. For accuracy, two dilution steps are recommended, as follows:
1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent **= [1:10k]**.

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up
- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for 5 to 30 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]
- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]
- Add 100ul of diluted Anti-Rat CRP-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]
- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.
  Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, assuring the top standard does not surpass 2 OD.

5. Stop Step [Stop: 100ul]
- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading
- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.