

High sensitivity **CRP**

A double antibody sandwich enzyme immunoassay for C-reactive protein

INTENDED USE

An enzyme immunoassay to measure levels of C-reactive protein (CRP) in human serum or plasma.

For research use.

INTRODUCTION

CRP is produced in the liver and is found only in low levels in normal serum. Elevated levels are found during acute infection, tissue injury and inflammatory disease. CRP is capable of activating both the opsonic and lytic potentials of the complement cascade.

Inflammation - and therefore elevated CRP levels - are associated with atherosclerosis. Myocardial infarction is often the end of a long process of atherosclerosis and it has recently been shown that moderately elevated levels of CRP are a significant risk factor.

Most commercially available CRP assays have been designed to measure the levels attained in overt inflammatory conditions so that these near-normal levels fall outside the quantitative range. In contrast, the Kalon Biological CRP EIA can be used to detect both low and high levels of CRP with a flexible dilution regime and does not require the use of a proprietary analyser system.

PRINCIPLE OF THE ASSAY

The assay is based on the double antibody sandwich format.

Polystyrene microtitre plate wells are supplied pre-coated with anti-CRP antibodies. Diluted serum samples and CRP standards are incubated in these wells, during which CRP is captured. After a wash step, the surface is probed for bound CRP with an anti-CRP antibody-enzyme conjugate. Following a second wash step, a chromogenic enzyme substrate is added to the wells. The reaction is halted by the addition of EDTA. The optical density is related to the level of CRP. CRP concentrations are read off the standard curve and, when corrected by the dilution factor, give the CRP concentration in the serum sample.

KIT PRESENTATION	1 plate	5 plate
<input type="checkbox"/> Antibody coated microtitre wells 8 well strips coated with affinity purified sheep anti-CRP	96 wells	480 wells
<input type="checkbox"/> Pre-diluted CRP standards Set of six, nominally containing 0, 1, 3, 10, 30 and 100 µg/L CRP (exact value printed on vial)	400 µL	1 mL
<input type="checkbox"/> CRP Tracer Affinity purified sheep anti-CRP labelled with alkaline phosphatase.	12 mL	60 mL
<input type="checkbox"/> Assay Diluent Buffered saline with protein stabiliser, surfactant and 0.1 g/L Thiomersal preservative	185 mL	2 × 500 mL
<input type="checkbox"/> Wash Concentrate (x40) Buffered saline and surfactant	50 mL	125 mL
<input type="checkbox"/> Substrate tablets 4-nitrophenylphosphate	1 × 20 mg	5 × 20 mg
<input type="checkbox"/> Substrate Buffer Contains sodium azide <10 g/L	12 mL	5 × 12 mL
<input type="checkbox"/> Stop Solution 50 g/L EDTA Solution.	15 mL	60 mL
<input type="checkbox"/> Resealable plastic bags	2	6

Store reagents refrigerated. Do not use beyond the expiry date printed on the label.

ADDITIONAL REQUIREMENTS

- Precision micropipettes to cover the range 10 to 1000 µL and preferably multichannel pipettes for volumes of 50 and 100 µL.
- Vortex mixer
- Microtitre plate shaker.
- Microtitre plate washer.
- Microtitre plate photometer with a 405 nm filter.
- Clean volumetric laboratory plastic or glassware.
- 1 mL microtitre plate-compatible dilution tubes.
- Purified water
- Quality control samples.

PROCEEDURAL NOTES

The standard curve covers the range 0 to 100 µg/L CRP. The normal range for CRP in serum or plasma is typically 200 to 6000 µg/L (0.2 to 6 mg/L), whilst the level in patients with inflammatory conditions may be 10 or 100 times the normal level. To measure CRP across this wide range, specimens can be diluted anywhere from 1/100 to 1/10,000 in Assay Diluent.

A dilution of 1 in 1,000 is appropriate for most specimens; samples giving results above the top standard can be re-assayed at 1 in 4,000 or 1 in 10,000.

ASSAY PROCEDURE

Preparation

- 1) Prepare an appropriate dilution of each specimen to be tested (see Procedural Notes above). For example,

To dilute 1 in 1,000 add 10 μ L sample to 490 μ L Assay Diluent and then dilute this by taking 10 μ L into 190 μ L Assay Diluent. Mix thoroughly at each stage.

To dilute 1 in 4,000 add 10 μ L sample to 490 μ L Assay Diluent and then dilute this by taking 10 μ L into 790 μ L Assay Diluent. Mix thoroughly at each stage.
- 2) Do not dilute the kit standards: they are already prepared to cover the assay range.
- 3) Prepare the required volume of working strength wash solution by diluting Wash Concentrate 1 in 40, e.g. make 25 mL Wash Concentrate to 1 L with purified water.
- 4) Prepare the required volume of substrate solution by adding one tablet of 4-nitrophenylphosphate to 12 mL substrate buffer and mixing to dissolve. Avoid contamination. Store unused substrate solution refrigerated for unused microtitre plate wells. A deep yellow colour prior to use indicates that the solution is contaminated and in this case should not be used.

Primary Incubation

- 5) Dispense 50 μ L of each CRP standard and diluted test sample into designated microwells.
- 6) Mix briefly on a plate shaker then cover and incubate the plate at room temperature for 60 minutes.
- 7) Wash the microwells -

Alternately fill and aspirate the microtitre wells with 350 μ L working-strength wash solution a total of four times. Tap out residual wash solution on clean absorbent towelling ready for the next step.

Secondary Incubation

- 8) Dispense 100 μ L CRP Tracer.
- 9) Mix briefly on a plate shaker then cover and incubate the plate at room temperature for 60 minutes
- 10) Wash the microwells as before.

Enzyme Incubation

- 11) Dispense 100 μ L substrate solution.
- 12) Mix briefly on a plate shaker, then incubate *uncovered* at room temperature for 30 minutes.
- 13) Dispense 100 μ L Stop Solution into each microwell.

Assay Completion

- 14) Read the microtitre well optical densities at 405 nm
- 15) Construct a standard curve using all the standard points. It is recommended to fit the curve using four parameter logistic curve fitting software.
- 16) Verify the assay.

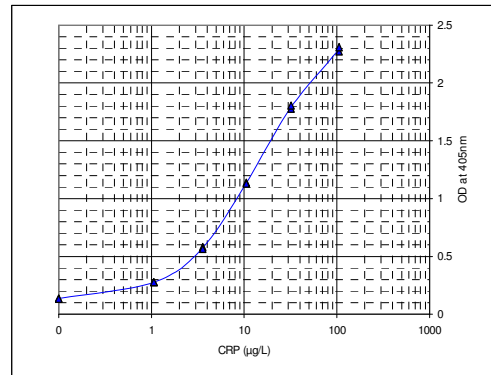
The assay is considered to have been executed correctly when the procedure has been followed correctly and the optical density for the 100 μ g/L standard is greater than 1.5 and less than 0.2 for the 0 μ g/L standard.

Control sera values should fall within the confidence range established in each laboratory

- 17) Read test sera values off the standard curve and correct for the serum dilution used.

EXPECTED VALUES

A typical standard curve



The CRP level was determined in Liquichek Immunology Controls lot 52110 from Bio-Rad Laboratories, Ca, USA.

Control	CRP (mg/L)
52111 - Level 1	16.5
52112 - Level 2	27.9
52113 - Level 3	35.6

The CRP level was determined in plasma collected from 32 normal volunteer blood donors in the UK. The following results were obtained.

Parameter	CRP (mg/L)
2.5 th percentile	0.20
Median	1.61
97.5 th percentile	5.48

BIBLIOGRAPHY

Pepys MB. C-reactive protein fifty years on. *Lancet* 1981;**1(8221)**:653-657.

Thomson SG *et al.* Hemostatic factors and the risks of myocardial infarction or sudden death in patients with angina pectoris. *New England Journal of Medicine* 1995;**332**:635-640.

Haverkate F *et al.* Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997;**349(9050)**:462-466.

Ridker PM *et al.* Inflammation, aspirin and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine* 1997;**336**:973-979.

Koenig W *et al.* C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Results from the MONICA Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999;**99**:237-242.

Ridker PM *et al.* C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine* 2000;**342**:836-843.

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