



# INSTRUCTION MANUAL

REF 4100

November 13<sup>th</sup> 2007

## Human CRP

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of C-reactive protein in human serum

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



**GA GENERIC ASSAYS GmbH**

Ludwig-Erhard-Ring 3  
15827 Dahlewitz, Germany

Telephone: +49 (0) 33708 – 9286 – 0  
Fax: +49 (0) 33708 – 9286 – 50

[www.genericassays.com](http://www.genericassays.com)

### INTENDED USE

Human CRP is used for the high sensitive quantitative determination of C-reactive protein (CRP) in human serum.

CRP an acute phase protein is produced and secreted by liver cells upon stimulation by pro-inflammatory cytokines such as interleukin 6. After stimulation CRP concentrations in serum may increase within few hours. Unlike other acute phase proteins giving hardly a doubling in their serum level CRP can rise 100 to 1000 times of the normal level within 24 h. CRP consisting of five non-glycosylated subunits was found at a molecular weight of 118 to 120 kDa. CRP precipitates un-specifically C-pneumococcal polysaccharides, opsonizes bacteria und activates the complement system.

Serum CRP concentrations of healthy individuals are found between 0 and 5 mg/L. Elevated serum CRP levels are an indicator for an acute or chronic inflammation observed e.g. during bacterial infections. Elevated serum CRP concentrations are found in patients suffering from autoimmune or immune complex disorders, tissue damage or malignant disease. The higher the CRP level the more tissue is inflamed and the higher the extend of inflammation. In terms of disease the inflammation process may be primary (rheumatoid arthritis) or secondary (malignancy, myocardial infarction).

Elevated serum CRP may occur during coronary events with patients suffering from stable angina pectoris. The relative risk is three times increased in such patients having CRP concentrations >3.6 mg/L (1). The base-line serum concentration of CRP may predict the risk of future myocardial infarction and stroke. Individuals showing CRP values from 1.15 to 2.10 mg/L have a relative risk of 2.6 and those with CRP values >2.10 mg/L one of 2.9, respectively (2).

Latest studies suggest that measurement of high sensitive CRP may strongly predict a pre-clinical phase of rheumatoid arthritis (RA) and may identify patients with RA and Systemic Lupus erythematoses (SLE) at greater risk for coronary calcium and cardiovascular mortality (3).

(1) Haverkate F, Thompson SG, Pyke SDM, Galimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. Lancet 1997 (349) 462-6

(2) Ridker PM, Cushman M.; Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997 (336) 973-9

(3) 2003 Annual ACR Meeting, Orlando, October 23-28, 2003, Abstracts

### PRINCIPLE OF THE TEST

Human CRP is an enzyme immunoassay for the quantitative determination of CRP in human serum.

CRP of the standards, control and diluted patient samples react with polyclonal anti-CRP antibodies, immobilized on the solid phase of microtiter plates. Anti-CRP antibodies highly purified by ion exchange chromatography guarantee the specific binding of CRP of the specimen under investigation. Following an incubation period of 30 min at 37°C unbound serum components are removed by a wash step.

The bound CRP react specifically with polyclonal anti-CRP antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37°C. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H<sub>2</sub>SO<sub>4</sub>) into the wells after 15 min at room temperature (RT) turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of CRP bound. The standard curve is established by plotting the CRP concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The antibody concentration of the specimen is directly read off the standard curve.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, haemolytic or contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep them at -20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

**Note:** *Patient samples have to be diluted 1 + 100 (v / v), e.g. 5 µl sample + 0.5 ml sample diluent 1 (C), prior to the assay.*

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires -20 °C.

## TEST COMPONENTS FOR 96 DETERMINATIONS

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with polyclonal anti-CRP antibodies (sheep)	1 vacuum sealed with desiccant, 2 adhesive foils
<b>Ag</b> <b>96</b>		
<b>B</b>	<b>Concentrated wash buffer</b> sufficient for 1000 ml solution	100 ml concentrate capped white
<b>BUF</b> <b>WASH</b>	<b>10x</b>	
<b>C</b>	<b>Sample diluent 1</b> blue coloured	50 ml ready for use capped green
<b>DIL 1</b>		
<b>D</b>	<b>Sample diluent 2</b> red coloured	11 ml ready for use capped green
<b>DIL 2</b>		
<b>E</b>	<b>Conjugate</b> containing polyclonal anti-CRP antibodies (sheep) coupled with HRP	11 ml ready for use capped red
<b>CONJ</b>		
<b>F</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	11 ml ready for use capped blue
<b>SOLN</b> <b>TMB</b>		
<b>G</b>	<b>Stop solution</b> 0.25 M sulphuric acid	15 ml ready for use capped yellow
<b>H2SO4</b> <b>0.25M</b>		
<b>1 - 5</b>	<b>Standards</b> (diluted serum) conc.: see leaflet enclosed	0,5 ml each ready for use
<b>CAL</b>		
<b>P</b>	<b>Positive control</b> (diluted serum) conc.: see leaflet enclosed	0,5 ml ready for use
<b>CONTROL</b>		

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 5 - 10 µl
- disposable pipette tips
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- glassware
- microplate reader with optical filters for 450 nm and 620 or 690 nm
- distilled or de-ionized water

### Size and storage

Human CRP has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Human CRP have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

### Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.

For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- **Dilute patient sera with sample diluent 1 (C) 1 + 100 (v / v), e.g. 5 µl serum + 0.5 ml sample diluent 1 (C).**
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense 100 µl sample diluent 2 (D) into the respective wells.
3. Add  
**25 µl** standards (1 - 5)  
**25 µl** positive control  
**25 µl** diluted patient samples  
into the respective wells.
4. Incubate **30 min** at 37°C.
5. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
6. Add **100 µl** of conjugate (E) solution to each well.
7. Incubate **30 min** at 37°C.
8. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
9. Add **100 µl** of substrate (F) to each well.
10. Incubate **15 min protected from light** at room temperature (18-25°C).
11. Add **100 µl** of stop solution (G) to each well and mix gently.
12. Read the OD at **450 nm** versus 620 or 690 nm within **30 min after adding the stop solution.**

## DATA PROCESSING

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the standards 1 - 5 on the ordinate, y-axis, (lin. scale) versus their respective CRP concentrations on the abscissa, x-axis, (log. scale).

CRP concentrations of the unknown samples are directly read off in mg/L against the respective OD values.

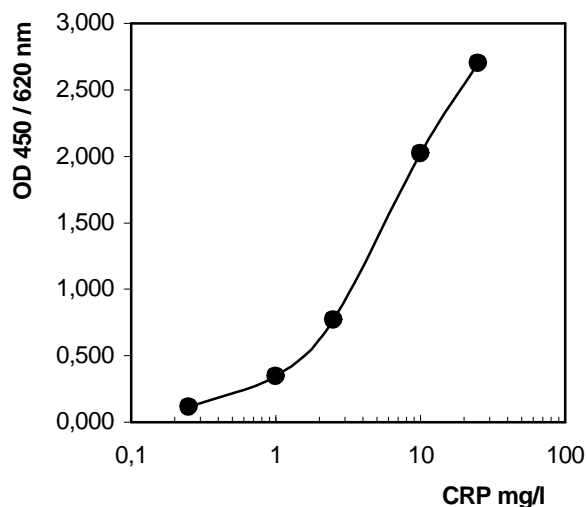
Human CRP may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

### Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	mg/L
Calibrator 1	0,109	0,123	0,116	<b>0,25</b>
Calibrator 2	0,355	0,341	0,348	<b>1,0</b>
Calibrator 3	0,792	0,753	0,772	<b>2,5</b>
Calibrator 4	2,011	2,035	2,023	<b>10,0</b>
Calibrator 5	2,687	2,717	2,702	<b>25,0</b>
Patient 1	1,813	1,799	1,806	7,3

### TYPICAL STANDARD CURVE



Specimens with an OD > standard 5, should be diluted with CRP negative serum and tested again. Results are multiplied with the dilution factor chosen.

### Test validity

The test run is valid if:

- the mean OD of the standard 1 is  $\leq 0.4$
- the mean OD of the standard 5 is  $\geq 1.2$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

## REFERENCE VALUES

Human CRP	mg/l
Normal range	$\leq 5.0$

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum CRP levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

### Limitations of Method

As the absorbance values could vary from test to test, the standard curve has to be included in every test run.

Contaminated test reagents as well as contaminated samples can cause false results. Also cross contaminations of the kit reagents and samples can cause false results.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

## CHARACTERISTIC ASSAY DATA

### Calibration

Human CRP is calibrated against the international reference for human serum proteins CRM 470.

### Linearity

Defined dilutions of the reference material with CRP free human serum are found congruent to calculation with the Human CRP. Dilutions of specimen in CRP free human serum are determined according to their expected theoretical values by the Human CRP.

### Sensitivity

The analytical sensitivity of this assay was determined at 0.07 mg/ml.

### Precision

Intraassay variability n = 22

Sample	Mean mg/l	SD	CV (%)
2	0.50	0.02	3.6
3	2.08	0.05	2.4
4	4.57	0.21	4.6
5	17.70	1.33	7.5

Interassay variability n = 22

Sample	Mean mg/l	SD	CV (%)
1	0.59	0.03	5.1
2	2.21	0.15	6.8
3	4.45	0.25	5.6
4	17.69	1.12	6.3

## INCUBATION SCHEME

# Human CRP (4100)

<b>Dilute patients sample</b>	<b>5 µl serum + 0.5 ml Sample diluent 1 (C)</b>
-------------------------------	---

1	<b>Bring all reagents to room temperature (18 - 25 °C)</b>			
2	Pipette Sample diluent 2 (D)	100 µl	100 µl	100 µl
3	Pipette Standards (1 - 5) Positive Control (P) 1 + 100 prediluted sera	25 µl	25 µl	25 µl
4	Incubate 30 min, 37°C			
5	Wash Decant, 3 x 300 µl (made of B)			
6	Pipette conjugate (E)	100 µl	100 µl	100 µl
7	Incubate 30 min, 37°C			
8	Wash Decant, 3 x 300 µl (made of B)			
9	Pipette substrate (F)	100 µl	100 µl	100 µl
10	Incubate protected from light 15 min, room temperature (18-25°C)			
11	Pipette stop solution (G)	100 µl	100 µl	100 µl
12	Read at 450 nm against 620 (690) nm within 30 min.			

## SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.