

## Instructions for Use

# CRP ELISA

Enzyme immunoassay for the quantitative determination of C-reactive protein in human serum and plasma.

**REF** **EU59131**

 **96**

   **2°C** **8°C**

EU: **IVD** **C** **E**



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## 1. INTENDED USE

Enzyme immunoassay for the quantitative determination of C-reactive protein in human serum and plasma.

## 2. SUMMARY AND EXPLANATION

C-Reactive Protein (CRP) is an acute-phase protein, produced exclusively in the liver. Interleukin-6 is the mediator for the synthesis by the hepatocytes of CRP, a pentamer of approximately 120.000 Daltons. CRP is present in the serum of normal persons at concentrations ranging up to 5 mg/L. The protein is produced by the foetus and the neonate and it does not pass the placental barrier, as such it can be used for the early detection of neonatal sepsis.

Because febrile phenomena, leukocyte count and erythrocyte sedimentation rate (ESR) are often misleading, investigators and clinicians now prefer a quantitative CRP determination as a marker for acute inflammation and tissue necrosis. Within 6 hours of an acute inflammatory challenge the CRP level starts to rise.

Serum concentration of CRP increases significantly in cases of both infectious and non-infectious inflammation, of tissue damage and necrosis and in the presence of malignant tumours. CRP is present in the active stages of inflammatory disorders like rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome, psoriatic arthropathy, systemic lupus erythematosus, polyarteritis, ulcerative colitis and Crohn's disease.

Injuries causing tissue breakdown and necrosis are associated with increases in serum CRP which are seen in thermal burns, major surgery and myocardial infarction.

Widespread malignant disease with carcinoma of the lung, stomach, colon, breast, prostate and pancreas, Hodgkin's disease, non-Hodgkin's lymphoma and lymphosarcoma will give rise to high levels of CRP resulting from tissue damage by invading tumour cells. CRP, therefore may be used to monitor malignancy.

The CRP-level increases dramatically following microbial infections, and this may be particularly helpful for the diagnosis and monitoring of bacterial septicemia in neonates and other immunocompromised patients at risk. In children, CRP is useful for differential diagnosis of bacterial and viral meningitis.

Because the biological half-life of this protein is only 24 hours, CRP accurately parallels the activity of the inflammation process and the CRP concentration decreases much faster than ESR or any other acute phase parameter, which is particularly useful in monitoring appropriate treatment of bacterial diseases with antibiotics.

C-Reactive Protein measurements during the early and late post-transplant period of bone marrow and organ transplantations is particularly useful in the management of interfering infections in these immunosuppressed patients.

## 3. TEST PRINCIPLE

Microtiter strips coated with anti-CRP antibody are incubated with diluted standards and patient samples. During this incubation step CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies.

After removal of the unbound conjugate, the strips are incubated with a chromogen solution containing tetramethylbenzidine and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H<sub>2</sub>SO<sub>4</sub> and the absorbance values at 450 nm are determined.

A standard curve is obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in patient samples is determined by interpolation from the standard curve.

**4. REAGENTS**

1. **MTP** **Microtiter Plate**  
12 x 8-well strips coated with monoclonal antibodies to human CRP.
2. **ENZCONJ** **Enzyme Conjugate**  
1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 mL). Contains antimicrobial agents and an inert red dye.
3. **CAL A-E** **Standard A-E**  
5 vials, each containing 1/10 prediluted CRP standard solutions (0.2 mL):  
0; 5; 25; 50; 100 µg/mL. Contain 0.09 % NaN<sub>3</sub>.  
Calibrated against the NIBSC 1st International Standard, 85/506 (specification 90-110% agreement)
4. **SAMPLEDIL** **CONC** **Sample Diluent Buffer**  
1 vial, containing 40 mL dilution buffer 5x concentrated.  
Contains 0.09 % NaN<sub>3</sub> and an inert green dye.
5. **TMB SUBS** **TMB Substrate Solution**  
1 vial, containing 15 mL of a solution containing H<sub>2</sub>O<sub>2</sub> and tetramethylbenzidine.
6. **TMB STOP** **TMB Stop Solution**  
1 vial, containing 12 mL of 0.5M H<sub>2</sub>SO<sub>4</sub>
7. **WASHBUF** **CONC** **Wash Buffer**  
1 vial containing 50 mL 20 x concentrated phosphate buffered washing solution.

**5. MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiter plate reader capable of measuring absorbance at 450 nm

**6. WARNINGS AND PRECAUTIONS FOR USERS**

1. For in vitro diagnostic use only.
2. For professional laboratory use.
3. Human blood components used in the preparation of the standards have been tested and found to be nonreactive for hepatitis B surface antigen and HIV I. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standards in the same way as potentially infectious material.
4. Dispose of patient samples and all residual products, containers and residues from tests using these reagents as if contaminated with potentially infectious substances. Safe disposal of residual products and their containers or packaging and residues from tests using these reagents must be in accordance with hospital policies and local and/or national legislation.
5. Do not mix reagents or coated microtiter strips from kits with different lot numbers.
6. Chromogen Solution / TMB Substrate Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0,3 %. It is classified as a Reproductive Toxicant Category 1B.  
Following hazard statements are applicable:  
H360D: May damage the unborn child.  
Following precautionary statements are applicable:  
P280: Wear protective gloves/protective clothing/eye protection/face protection.  
P308+P313: If exposed or concerned: Get medical advice/attention.
7. Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.
8. Serious incidents related to the CRP ELISA must be reported to the manufacturer and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. STORAGE CONDITIONS

1. Store at 2 - 8°C.
2. Store the Microtiter Plate in their original package with the desiccant until all the strips have been used. Opened components should be stored at 2-8°C until next use and can be maintained for 6 months.
3. Never use any kit components beyond the expiration date.

## 8. SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay. Remove serum from clot as soon as possible to avoid haemolysis. Lipemic and/or haemolysed samples can cause false results. Transfer the serum to a clean storage tube. Specimens may be stored at 2 - 8°C for a few days, (3 days)<sup>[1]</sup> or they can be stored frozen for a longer period of time (6 months at -20°C, indefinitely at -70°C)<sup>[1]</sup>. Avoid repeated freezing and thawing.

## 9. ASSAY PROCEDURE

### 9.1. General Remarks

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. If an ELISA Washer is used, adaptation of the washing step might be necessary to obtain optimal results.

### 9.2. Reconstitution of the Reagents

Wash Buffer: dilute 50 mL of concentrated Wash Buffer to 1000 mL with distilled water. Reconstituted solution can be stored at least 1 month store at 2 - 8°C.

At higher temperatures, the concentrated Wash Buffer may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

Sample Diluent Buffer: Dilute 40 mL of the concentrated Sample Diluent Buffer to 200 mL with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear. Store at 2 - 8°C.

### 9.3. Assay Procedure

1. The 10 x prediluted standards are diluted **1:100** as follows: pipette **10 µL** of each **calibrator** into separate glass or plastic dilution tubes. Add **990 µL** of **diluted Sample Diluent Buffer** and mix carefully.
2. The patient samples are **diluted 1:1000** in two consecutive steps: pipette **10 µL** of each **patient sample** into separate glass or plastic dilution tubes and add **990 µL** of **diluted Sample Diluent Buffer**. Mix thoroughly. Add **450 µL** of **diluted Sample Diluent Buffer** to **50 µL** of these **100 x prediluted samples**. Mix thoroughly.  
**Warning: do not store the diluted samples for more than 8 hours.**
3. Pipette **100 µL** of the **diluted calibrators and samples** into each of a pair of adjacent wells.
4. Incubate the covered Microtiter Plate for **30 ± 2 min** at **room temperature**.
5. Wash the Microtiter Plate **three times** with **Wash Buffer**. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in Wash Buffer. During the third step, the Wash Buffer is left in the strips for 2-3 min. Change Wash Buffer for each cycle. Finally empty the Microtiter Plate and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add **100 µL** of **Enzyme Conjugate** and incubate the covered Microtiter Plate for **30 ± 2 min** at **room temperature**.
7. Repeat the washing procedure as described in 5.
8. Add **100 µL** of **TMB Substrate Solution** to each well.
9. Incubate for **10 ± 2 min** at **room temperature**. Avoid light exposure during this step.
10. Add **50 µL** of **TMB Stop Solution** to each well.
11. Determine the absorbance of each well at 450 nm or at 450 nm with reference filter 600-650 nm **within 30 min** following the addition of acid.

## 10. RESULTS

The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. linear/linear; log/linear) is constructed.

Use the average absorbance of each patient sample obtained in the CRP ELISA to determine the corresponding value by simple interpolation from the curve.

Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.

When using the sample dilution 1:1000, results are directly read by interpolation from the standard curve, and values are expressed as µg/mL.

When using a higher dilution, results must be multiplied by a factor equal to the ratio to 1000. Example, if a 1:2000 sample dilution is used, results must be multiplied by 2 and values are expressed as µg/mL.

When using a lower dilution, results must be divided by a factor equal to the ratio to 1000. Example, if a 1:500 sample dilution is used, results must be divided by 2 and values are expressed as µg/mL.

## 11. PERFORMANCE CHARACTERISTICS

### 11.4. Example of typical OD values:

Standard (µg/mL)	OD value
0	0.019
5	0.240
25	0.821
50	1.301
100	2.018

### 11.5. Normal Values:

Serum and plasma samples from 360 healthy blood donors were tested with the CRP ELISA, the following distribution of CRP levels has been found:

Concentration Range CRP µg/mL	Number of donors
≤ 5	328
6-25	31
26-50	1
> 50	0

All individuals have small amounts of CRP in their blood. The upper limit of the normal range is situated between 5 and 8 µg/mL (343 donors in the population of 360 individuals had CRP levels < 8 µg/mL).

### 11.6. Precision

Precision has been determined by assaying three spiked samples in a study based on CLSI EP05-A3 guidance document (ANOVA analysis).

Repeatability (replicate-to-replicate variability) and within laboratory precision (run-to-run and day-to-day variability) has been assessed in a 20 x 1 x 5 study design (20 days, 1 run/day, 5 replicates/run (n = 100 for each sample)), performed by one operator for one batch of the CRP ELISA.

Obtained results:

Sample	Mean (µg/mL)	Repeatability		Within-Laboratory Precision	
		SD (µg/mL)	CV (%)	SD (µg/mL)	CV (%)
Level 1	3.47	0.271	7.8	0.323	9.3
Level 2	31.51	1.481	4.7	2.962	9.4
Level 3	75.01	4.276	5.7	8.776	11.7

Reproducibility (site-to-site and instrument-to-instrument variability) has been determined based on a 3 x 5 x 1 x 5 study design (3 sites, 5 days, 1 run/day, 5 replicates/run (n = 75 for each sample)) performed by three operators, with three instruments, at three sites for one batch of the CRP ELISA.

Obtained results:

Sample	Mean (µg/mL)	Reproducibility	
		SD (µg/mL)	CV (%)
Level 1	3.54	0.521	14.7
Level 2	33.19	3.983	12.0
Level 3	75.71	11.963	15.8

## 12. ANALYTICAL SPECIFICITY

### 12.7. Cross-reactivity

The CRP ELISA recognizes natural and recombinant human CRP. No cross-reactivity was observed with following factors, prepared at 1 µg/mL in sample diluent: human pentraxin 2; human pentraxin 3; human monomeric CRP; rat CRP.

### 12.8. Endogenous Interference

Potential interference of following endogenous factors was investigated, according to the CLSI guideline EP-07. No interference was found for hemoglobin (5 mg/mL) lipids (intralipid, 2.5 mg/mL), bilirubin (0.2 mg/mL) and RF (250 IU/mL).

### 12.9. Detection Capability

The characteristics Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) have been determined according to the CLSI Guideline EP17-A2, whereby for LoB and LoD determination the classical approach has been followed and for LoQ determination the precision profile approach.

Following results are obtained:

LoB: 0.052 µg/mL

LoD: 0.225 µg/mL

LoQ: 0.586 µg/mL

### 12.10. Method Comparison

A clinical sample panel (38 serum samples) has been analysed on the CRP ELISA and a commercial assay on the market (CRP Vario, Sentinel Diagnostics). Pearson r correlation was 0.999 (CI 95%: 0.999 - 1.000). Passing-Bablok regression analysis revealed Y-intercept -0.32 (CI 95%: -0.48 - 0.12) and slope 0.98 (CI 95%: 0.942 - 1.022).

## 13. TEST VALIDITY

The following specifications must be met for each run to be valid:

OD value for the zero calibrator: < 0.080

OD value for the highest value calibrator: > 1.000

If one of the specifications is not met, the test run should be repeated.












## 14. TROUBLE SHOOTING

In case of high background signal, the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

## 15. REFERENCES

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2. POWELL L. J. C-Reactive Protein - a Review Am. J. Med. Technol., 87, 138-142 (1979).
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# Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.-Cat.: / N.º Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλοσμένο
	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
	Unique Device Identification / Eindeutige Geräteerkennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazena a: / Conservare a: / Armazena em: / Αποθήκευση στους:
	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazena a: 2 - 8°C / Conservare a: 2-8°C / Armazena em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symboles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του kit συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.

For further country-specific classifications, please refer to the corresponding safety data sheet.



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