Instructions for Use

CRP ELISA

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

REF **EU59131**

Σ **96**

2-8°C

EU: IVD CE

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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 5mg/l. The protein is produced by the fetus and the neonate and it does not pas the placental barrier, as such it can be used for the early detection of neonatal sepsis.
Because febrile phenoena, leukocyte count and erytrhocyte sedimentation rate (ESR) are often misleading, investigators and clinicans 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, psoriatric 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 ESR1,2 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

Microtiterstrips 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 tetramethylbenzidin 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_2SO_4 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 **Microtiterplate**
   12 x 8-well strips coated with monoclonal antibodies to human CRP.
2. 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. Calibrated against the NIBSC 1st International Standard, 85/506. Contain 0.09 % NaN_3 and antimicrobial agents as preservatives.
3. ENZCONJ **Enzyme Conjugate**
   1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 mL). Contains antimicrobial agents and an inert red dye.
4. SAMPLEDIL CONC **Sample Diluent Buffer**
   1 vial, containing 40 mL dilution buffer 5x concentrated. Contains 0.09 % NaN_3 and antimicrobial agents and an inert green dye.
5. WASHBUF CONC **Wash Buffer**
   1 vial containing 50 mL 20 x concentrated phosphate buffered Wash Buffer.
6. TMB SUBS **TMB Substrate Solution**
   1 vial, containing 15 mL of a solution containing H_2O_2 and tetramethylbenzidin.
7. TMB STOP **TMB Stop Solution**
   1 vial, containing 12 mL of 0.5M H_2SO_4

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 microtiterplate reader capable of measuring absorbencies at 450 nm
5. Vortex mixer
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

6. WARNINGS AND PRECAUTIONS FOR USERS

1. For in vitro diagnostic use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
7. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. 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. Dispose patient samples and all materials used to perform this test as if they contain infectious agents.
10. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
11. 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.

7. STORAGE CONDITIONS

1. Store at 2-8 °C.
2. Store the microtiterstrips in their original package with the desiccant until all the strips have been used.
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, or they can be stored frozen for a longer period of time. Avoid repeated freezing and thawing.

9. ASSAY PROCEDURE

9.1. Allgemeine Bemerkungen

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
3. Use a pipetting scheme to verify an appropriate plate layout.
4. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration.
5. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells
6. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
7. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
8. Once the assay has been started, all steps should be completed without interruption.
9. 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 or as long as solution remains clear. 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 microtiterstrips for **30 ± 2 min** at **room temperature.**
5. Wash the microtiterstrips **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 microtiterstrips and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add **100 µL** of **Enzyme Conjugate** and incubate the covered microtiterstrips 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 **within 30 min** following the addition of acid.

10. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

11. RESULTS

The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. 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.

12. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences.
They have to be correlated to other clinical observations and diagnostic tests.

Example of typical O.D. values:

CALIBRATOR µg/mL	O.D. value
0	**0.019**
5	**0.240**
25	**0.821**
50	**1.301**
100	**2.018**

Normal Values:
Serum and plasma samples from 360 healthy donors were tested with the IBL 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).

13. PERFORMANCE

13.1. PRECISION

Intra Assay (n = 10)	**Level 1**	**Level 2**
Mean (µg/mL)	5.2	48.3
SD (µg/mL)	0.27	3.3
% CV	5.12	6.84

Inter Assay (n = 7)	**Level 1**	**Level 2**	**Level 3**
Mean (µg/mL)	4.3	31.0	67.2
SD (µg/mL)	0.6	3.6	8.5
% CV	14.3	11.6	12.7

14. SPECIFICITY

14.1. 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.

14.2. ANALYTICAL SENSITIVITY

The minimal detectable concentration is < 1 µg/mL.

14.3. TEST VALIDITY

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

O.D. value for the zero calibrator: < 0.080
O.D. value for the highest value calibrator: > 1.000

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

15. REFERENCES

1. POWELL L. J. C-Reactive Protein - a Review Am. J. Med. Technol., **87**, 138-142 (1979).
2. GEWURZ H., MOLD C., SIEGEL J. and FIEDEL B. C-Reactive Protein and the Acute Phase Response Advances in Internal Medicine, **27**, 345-372 (1982).
3. HELGESON N. G. P., ADAMSON D. M., PIKE R. B., JAMES D. S., NICODEMUS D. S., LEE B. A. and MILLER G. W. C-Reactive Protein : Laboratory Medicine, Vol. 2 (Race G. J., Ed.), Harper & Row, Hagerstown, chapter 29 (1973).
4. Johnson HL., Chiou CC., Cho CT. Applications of acute phase reactants in infectious diseases J. Microbiol. Immunol. Infect. 32(2):73-82 (1999).

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / 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. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / 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 symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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.


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