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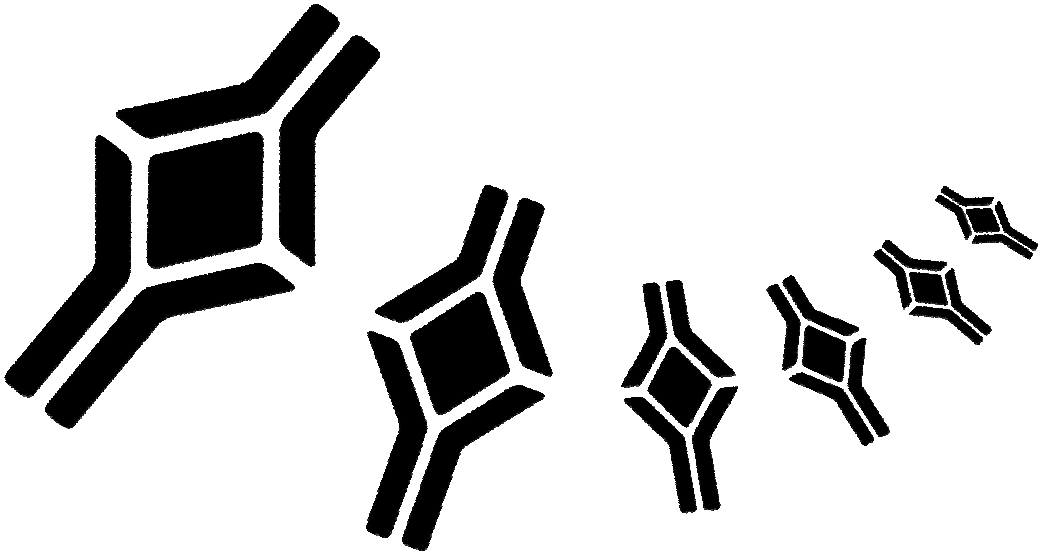

Human CRP ELISA

Product Data Sheet

Cat No.: RAP001

European Union: IVD CE

Rest of the world:
For research use only!

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» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

Enzyme Immunoassay for the Quantitative Determination of C-Reactive Protein in Human Serum and Plasma.

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 foetus 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 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, 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 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.

The CRP ELISA is an enzyme immunoassays for the quantitative determination of C-Reactive Protein in human serum and plasma.

2. PRINCIPLE OF THE CRP ELISA

Microtiterstrips coated with anti-CRP antibody are incubated with diluted standard sera 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.

3. REAGENTS

1. Coated Microtiterstrips - 12 x 8-well strips coated with monoclonal antibodies to human CRP.
2. Standard Sera - 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. Conjugate - 1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12 ml). Contains antimicrobial agents and an inert red dye.
4. Specimen Dilution Buffer - 1 vial, containing 40 ml dilution buffer 5x concentrated. Contains 0.09 % NaN_3 and antimicrobial agents and an inert green dye.
5. Washing Solution - 1 vial containing 50 ml 20 x concentrated phosphate buffered washing solution.
6. Chromogen Solution: 1 vial, containing 15 ml of a solution containing H_2O_2 and tetramethylbenzidin.
7. Stopping Solution - 1 vial, containing 12 ml of 0.5M H_2SO_4

4. MATERIALS REQUIRED BUT NOT SUPPLIED

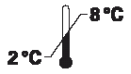
1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass tubes for the dilution of the samples.
4. A microtiterplate reader capable of measuring absorbencies at 450 nm

5. WARNINGS AND PRECAUTIONS FOR USERS

1. For in vitro diagnostic use only.
2. Human blood components used in the preparation of the standard sera 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 standard sera 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.
3. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
4. 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.

6. STORAGE CONDITIONS

1. Store the microtiterstrips in their original package with the desiccant until all the strips have been used.
2. Never use any kit components beyond the expiration date.



7. 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.

8. ASSAY PROCEDURE

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.

Reconstitution of the Reagents

Washing Solution: dilute 50 ml of concentrated Washing Solution (**5**) 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 Washing Solution (**5**) may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.
Sample diluent
Dilute 40 ml of the concentrated Sample Diluent 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.

Assay Procedure

1. The 10 x prediluted standard sera (**2**) are diluted 1:100 as follows : pipette 10 µl of each calibrator into separate glass dilution tubes. Add 990 µl of diluted Specimen Dilution Buffer (**4**) 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 dilution tubes and add 990 µl of diluted Specimen Dilution Buffer (**4**). Mix thoroughly. Add 450 µl of diluted Specimen Dilution 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 (**1**).
4. Incubate the covered microtiterstrips for 30 ± 2 min at room temperature.
5. Wash the microtiterstrips three times with Washing Solution. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2-3 min. Change washing solution for each cycle. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add 100 µl of Conjugate Solution (**3**) 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 Chromogen Solution (**6**) to each well.
9. Incubate for 10 ± 2 min at room temperature. Avoid light exposure during this step.
10. Add 50 µl of Stopping Solution (**7**) to each well.
11. Determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

9. 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.

10. EXPECTED VALUES

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**

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.

11. 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

12. MINIMAL DETECTABLE CONCENTRATION

The minimal detectable concentration is < 1 µg/ml.

13. REFERENCES

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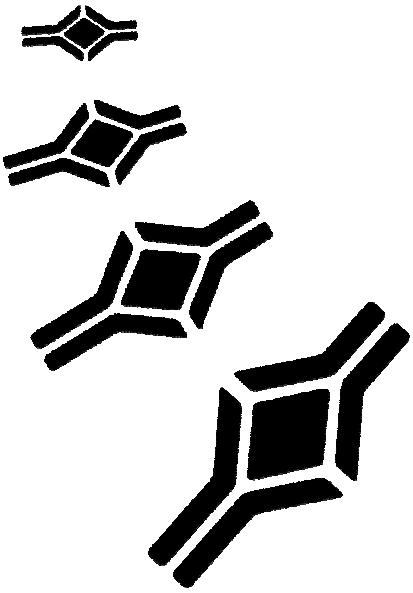

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