# hsCRP ELISA

Enzyme Immunoassays for the Quantitative High Sensitive 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.

A series of prospective studies provide consistent data documenting that mild elevation of baseline levels of CRP among apparently healthy individuals is associated with higher long-term risk for future cardiovascular events. This predictive capacity of CRP is independent of traditional cardiovascular risk factors and offers a prognostic advantage over measurement of lipid alone. Inflammatory markers specifically hsCRP may help to identify those who would benefit most from these pharmacological intervention. hsCRP is the novel and evolving biomarker which provides a most useful predictive indicator for subsequent cardiovascular events.
This test should not be used for assessment of acute inflammation but should be ordered to evaluate CVD (Cardiovascular Disease) risk in apparently healthy individuals who have not had recent infection or other serious illness.
For the assessment of acute inflammation the APDia CRP ELISA ref 740001 can be used.

## 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.

## REAGENTS

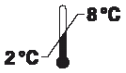
**1. Coated Microtiterstrips** - 12 x 8-well strips coated with monoclonal antibodies to human CRP.
**2. Standard Sera** - 5 vials, each containing 1/10 prediluted CRP standard solutions (0.2 ml): 0 - 0.4 - 1 - 5 - 10 µg/ml. Calibrated against the NIBSC 1st International Standard, 85/506. Contain 0,09 % $NaN_3$ 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 $H_2SO_4$

## 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

## 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.




## 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.

## 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.

## 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.
4. Absorbance is a function of the incubation time and temperature. Therefore the size of the assay run should be limited. It is suggested to run no more than 20 patient samples with one set of Reference Standards in duplicate.

**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.

## 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 hsCRP-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.

**EXPECTED VALUES**

**Example of typical O.D. values:**

| CALIBRATOR µg/ml | O.D. |
|---|---|
| **0** | **0.015** |
| **0.4** | **0.112** |
| **1** | **0.354** |
| **5** | **1.370** |
| **10** | **1.954** |

**Interpretation of the results**

The following criteria are commonly found in the literature for the relation between the CRP values and the risk for developing CVD.

CRP values < 1.0 mg/L = Low risk for CVD
CRP values 1.0 – 2.9 mg/L = Intermediate risk for CVD
CRP values > 3.0 mg/L = High risk for CVD

**PRECISION**

| *Intra Assay (n=10)* | **Level 1** | **Level 2** | **Level 3** |
|---|---|---|---|
| Mean (µg/ml) | 0.36 | 1.55 | 6.15 |
| SD (µg/ml) | 0.02 | 0.07 | 0.25 |
| %CV | 6.9 | 4.3 | 4.1 |
| *Inter Assay (n=24)* | **Level 1** | **Level 2** | **Level 3** |
| Mean (µg/ml) | 0.41 | 1.60 | 6.22 |
| SD (µg/ml) | 0.026 | 0.093 | 0.39 |
| %CV | 6.3 | 5.8 | 6.3 |

**MINIMAL DETECTABLE CONCENTRATION**

The minimal detectable concentration is approximately 0.02 µg/ml.

**REFERENCES**

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