

DRG® C-Reactive Protein (CRP) ELISA (EIA-1952)



Revised 1 Dec. 2010 rm (Vers. 9.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

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

1 PRINCIPLE OF THE CRP ELISA

Microtiterstrips coated with anti-CRP antibody are incubated with diluted standard sera and 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 0.5 M H₂SO₄ 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 samples is determined by interpolation from the standard curve.

2 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 % NaN₃ 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 % NaN₃ 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 H₂O₂ and tetramethylbenzidin.
7. **Stopping Solution** - 1 vial,
containing 12 ml of 0.5M H₂SO₄

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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

4 WARNINGS AND PRECAUTIONS FOR USERS

1. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
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 samples and all materials used to perform this test as if they contain infectious agents.
3. Do not mix reagents or coated microtiter strips 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.

5 STORAGE CONDITIONS

2 °C – 8 °C

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

6 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 °C – 8 °C for a few days, or they can be stored frozen for a longer period of time. Avoid repeated freezing and thawing.

7 ASSAY PROCEDURE**7.1 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.

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7.2 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 or as long as solution remains clear. Store at 2 °C - 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 °C - 8 °C.

7.3 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 samples are diluted **1:1000** in two consecutive steps:
pipette 10 µl of each 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 microtiter strips for 30 ± 2 min at room temperature.
5. Wash the microtiter strips 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 microtiter strips and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add 100 µl of Conjugate Solution (3) and incubate the covered microtiter strips 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.

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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 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.

9 REFERENCES / LITERATURE

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4. Johnson HL., Chiou CC., Cho CT. Applications of acute phase reactants in infectious diseases J. Microbiol. Immunol. Infect. **32**(2):73-82 (1999).