CRP ELISA Kit

(For Human)

Enzyme Immunoassay for the quantification of C-Reactive Protein (CRP) in serum and plasma

Catalog number: ARG80870

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CRP has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CRP present in samples is bound by the immobilized antibody. After washing away any unbound substances, an HRP conjugated antibody specific for CRP is added to each well and incubate. A substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of CRP bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of CRP in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standard 1-5	5 vials, each containing 1/10 prediluted CRP standard solutions (0.2ml each)	4°C
5X Specimen Dilution Buffer	1 vial (40 ml)	4°C
HRP-Conjugated antibody	1 vial (12ml) (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.

- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. Diluted wash buffer is stable for 30 days at 2-8°C.
- 1X Specimen Dilution Buffer: Dilute 40ml of the concentrated 5X buffer to 200ml distilled water. Diluted buffer is stable for 3 months at 2-8°C.
- Standards: The 1/10 prediluted standards are diluted 1:100 as follows: Pipette 10µl of each standard to 990µl 1X specimen dilution buffer in clean tubes. The final concentrations of each standard after dilution are: 0µg/ml, 5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml.
- Patient samples: Patient samples are to be diluted 1:1000 in two consecutive steps: Pipette 10µl of each patient sample to 990µl 1X specimen dilution buffer in clean tubes. Mix thoroughly. Add 50µl of the above-mentioned diluted sample to 450µl 1X specimen dilution buffer in clean tubes. Mix thoroughly. **Do not store the diluted samples for more than 8 hours.**

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Cover wells and incubate for 30 mins at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5

washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add 100 µl of HRP-conjugated antibody into each well.
6. Cover wells and incubate for 30 mins at RT.
7. Wash as according to step 4.
8. Add 100 µl of TMB Reagent to each well. Incubate for 10 minutes at room temperature in dark.
9. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

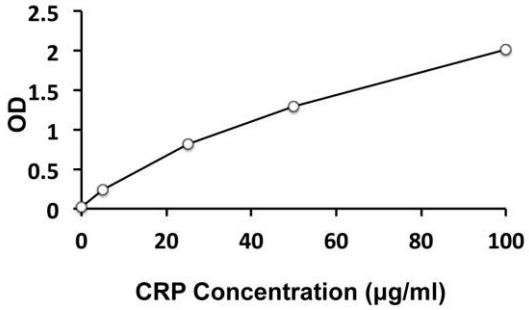
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter

Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of CRP ranged from 5-100 µg/ml. The mean MDD was 1 µg /ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.98% and inter-assay precision was 12.8%.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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