CRP

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C-Reactive Protein (ELISA)

REF: DS207713 Σ 96

Intended use
Immunoassay for the in vitro quantitative determination of CRP (C-Reactive Protein) in human serum and plasma.

Summary[1-6]
C-reactive protein (CRP) is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-membered ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever. In normal healthy individuals CRP is a trace protein with a range up to 5 mg/L. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease. Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis. Persistence of a high serum CRP concentration is usually a grave prognostic sign which generally indicates the presence of an uncontrolled infection.

Test principle
Sandwich principle. Total duration of assay: **40 minutes.**
- Sample, Anti-CRP coated microwells and enzyme labeled Anti-CRP are combined.
- During the incubation, CRP presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the CRP within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of CRP in the sample.

Reagents
Materials provided
- **Coated Microplate**, 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-CRP.
- **Calibrators**, 6 vials, 0.5 mL each, ready to use; Concentrations: 0(**A**), 5(**B**), 20(**C**), 50(**D**), 150(**E**) and 300(**F**) mg/L.
- **Enzyme Conjugate**, 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-CRP in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **Sample Dilute Concentrate,** 1 vial, 11 mL of serum diluent concentrate containing buffer containing 0.1% ProClin300 preservative
- **Substrate**, 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution**, 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate**, 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU**, 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 25 µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only. For professional use only.
- All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
- Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
- Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
- Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- If more than one plate is used, it is recommended to repeat the calibration curve.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Ensure that the bottom of the plate is clean and dry, and no bubbles are present on the surface of the liquid before reading the plate.
- The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Storage
- Store at 2-8°C.
- Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
- Seal and return unused calibrators to 2-8°C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20°C. Avoid multiple freeze-thaw cycles.
- Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum and plasma can 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
- Li-heparin, K2-EDTA and K3-EDTA plasma.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
- Centrifuge samples containing precipitates before performing the assay. Do not use samples and controls stabilized with azide.
- **Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25°C) before measurement.**
- Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
- Don's use Substrate if it looks blue.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Reagents Preparation
Dilution of Sample Dilute Concentrate
Dilute contents of sample diluent concentrate to 100 mL with distilled or deionized water in a suitable storage container. Store diluted solution at 2-8°C.

Test procedure
- ***Dilution of Patient Sample:*** Pipette **10 µL** of **patient sample** into separate glass or plastic dilution tubes, then add **990 µL** of **diluted Sample Diluent Concentrate** and mix carefully.
  Warning: do not store the diluted samples for more than 8 hours.

- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.

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- Add **100 µL** of **enzyme conjugate** to each well.
- Add **10 µL** of **calibrators** or **Diluted Patient Sample** to each well.
  Note: Don't dilute calibrators.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37°C** for **30 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µl** of **substrate** to each well.
- **Incubate** at **ambient temperature (18-25°C)** in the dark for reaction for **10 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in mg/L for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

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

Sample	Value (mg/L)	OD (450nm)
Calibrator A	0	0.012
Calibrator B	5	0.154
Calibrator C	20	0.407
Calibrator D	50	0.985
Calibrator E	150	1.711
Calibrator F	300	2.693
Control 1	37.39	0.742
Control 2	118.6	1.483
Sample	33.08	0.659

Limitations - interference
Results of studies show that the following substances do not interfere with this C-reactive protein procedure.
The criteria for no significant interference is recovery within 10% of the initial value.

Substances	Non-interfering concentrations (mg/L)
Bilirubin	< 600 µmol/L or < 35 mg/dL
Hemolysis	< 600 µmol/L or < 36 mg/dL
Lipemia	< 1200 mg/dL

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges
Measuring range
0.1 - 300 mg/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.1 mg/L. Values above the measuring range are reported as > 300 mg/L.

Lower limits of measurement
Lower detection limit
Lower detection limit: 0.1 mg/L
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values
Reference range
< 5.0 mg/L

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean mg/L	SD mg/L	CV %	SD mg/L	CV %
Human Serum 1	3.24	0.28	8.65	0.288	8.89
Human Serum 2	38.61	2.807	7.27	3.131	8.11
Human Serum 3	136.93	7.723	5.64	8.777	6.41
PC Universal 1	35.48	2.416	6.81	2.586	7.29
PC Universal 2	117.47	6.296	5.36	8.047	6.85

Method comparison
A comparison of the DiaSino CRP assay (y) with the Roche Tina-quan Gen.3 CRP assay (x) using 106 clinical serum samples gave the following correlations (mg/L): r = 0.9733

Functional sensitivity
0.2 mg/L
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

References

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Symbols

IVD In vitro diagnostic medical device	8°C / 2°C Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Contains sufficient for <n> tests
Manufacturer	Do not use if package is damaged and consult instructions for use		

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