MONKEY C-REACTIVE PROTEIN (CRP) ELISA
Life Diagnostics, Inc., Catalog Number: CRP-3

INTRODUCTION

CRP is an acute phase protein in monkeys that is elevated in serum because of injury, infection, or disease. Normal levels of CRP range from 0-8.3 µg/ml[1,2] and levels may increase 100-fold or more during the acute phase response.[1-8]

PRINCIPLE OF THE ASSAY

The assay uses affinity purified monkey CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated monkey CRP antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in CRP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If CRP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of CRP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- CRP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- CRP stock (lyophilized). **Store ≤ -20°C**
- 20x Wash solution: CRPW50-20, 50 ml
- 10x Diluent: YD25-10, 25 ml
- TMB: TMB11-1, 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:
- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The CRP stock should be stored at or below -20°C for optimum stability (it can be safely shipped at 4°C). The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.

3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The CRP stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved. Reconstituted stock should be aliquoted and stored frozen at or below -20°C (within 1 hour of reconstitution) if future use is intended.
2. Label 7 polypropylene or glass tubes as 75, 37.5, 18.75, 9.38, 4.69, 2.34 and 1.17 ng/ml.
3. In the tube labeled 75 ng/ml prepare the 75 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 37.5, 18.75, 9.38, 4.69, 2.34 and 1.17 ng/ml.
5. Prepare the 37.5 ng/ml standard by mixing 250 µl of the 75 ng/ml standard with 250 µl of diluent in the tube labeled 37.5 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

In studies at Life Diagnostics, we found that CRP levels ranged from approximately 5 µg/ml in normal serum to 150 µg/ml or greater in serum from sick monkeys. We suggest that samples initially be diluted 1000-fold using the following procedure for each sample to be tested.

1. Dispense 90 µl and 495 µl of 1x diluent into separate tubes for each sample to be tested.
2. Pipette and mix 10.0 µl of serum or plasma into the tube containing 90 µl of 1x diluent to give a 10-fold dilution.
3. Mix 5.0 µl of the 10-fold diluted sample with 495 µl of 1x diluent in the second tube to give a 1000-fold dilution.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.

4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 μl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 μl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CRP (ng/ml)	Absorbance (450 nm)
75	3.538
37.5	2.299
18.75	1.638
9.38	0.978
4.69	0.633
2.34	0.430
1.17	0.309
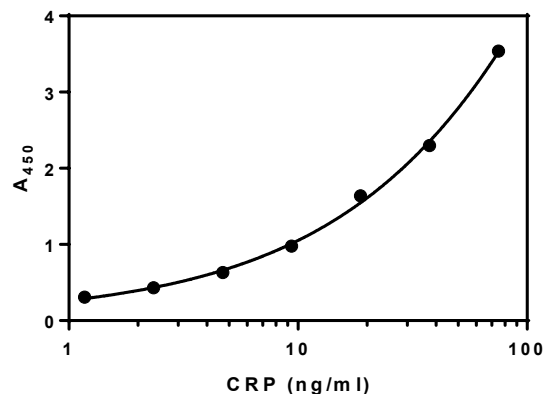
REFERENCES

1. Jinbo T, Hayashi S, Iguchi K, Shimuzu M, Matsumoto T, Naiki M, and Yamamoto S. Development of monkey C-Reactive protein method. Vet Immunol Immunopathol. 61:195-202 (1998)
2. Jinbo T, Ami Y, Suzaki Y, Kobune F, Ro S, Naiki M, Iguchi K, Yamamoto S. Concentrations of c-reactive protein in normal monkeys (Macac irus) and in monkeys inoculated with Bordella bronchiseptica R-5 and measles virus. Vet Res Commun. 23:265-74 (1999)
3. Hart BAT, Bank RA, De Roos JADM, Brok H, Jonker M, Theuns HM, Kakami J and Te Koppele JM. Collagen-induced arthritis in rhesus monkeys: evaluation of markers for inflammation and joint degradation. British J. Rheumatology 37:314-323 (1998)
4. Kingstrom J, Plyusnin A, Vaheri A and Lundkvist A. Wild-type Puumala Hanatavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in Cynomolgus Macaques. J. Virology. 76:444-449 (2002)
5. Ossetrova NI, et. al. The use of discriminant analysis for evaluation of early-response multiple biomarkers of radiation exposure using non-human primate 6Gy whole body radiation model. Radiation Measurements 42:1158-1163 (2007)
6. Blackwood RS, et. al. Effects of the Macrolide drug tylosin on chronic diarrhea in Rhesus Macaques (Maccaca mulatta). Comparative Medicine 58(1):81-87 (2008)
7. Blakely WF, et. al. Multiple parameter radiation injury assessment using a nonhuman primate radiation model-biodosimetry applications. Health Phys. 98(2):153-159 (2010)
8. Ossetrova NI, Sandgren DJ and Blakely WF. C-reactive protein and serum amyloid A as early-phase and prognostic indicators of acute radiation exposure in nonhuman primate total-body irradiation model. Radiation Measurements 46:1019-1024 (2011)

For technical assistance please email us at
techsupport@lifediagnostics.com