

MONKEY C-REACTIVE PROTEIN (CRP) ELISA TEST KIT

Life Diagnostics, Inc., Catalog Number: 2210-4

Enzyme Immunoassay for the Quantitative Determination of Monkey C-Reactive Protein (CRP) in Serum

****FOR RESEARCH USE ONLY****

PLEASE READ ATTACHED MSDS BEFORE USING THE KIT

INTRODUCTION

CRP is an acute phase protein in monkeys that is elevated in serum as a result of injury, infection or disease. Normal levels of CRP range from 0-8.3 $\mu\text{g/ml}$ ^{1,2} and levels may increase one hundred fold or more during the acute phase response¹⁻⁴.

PRINCIPLE OF THE TEST

The monkey CRP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-monkey CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-monkey CRP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and 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-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of CRP is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-monkey CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard containing monkey CRP (concentration and dilution instructions are listed on the vial label)

Store at -20°C.

- 10x Diluent (25 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer

- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

The lyophilized reference standard should be stored at or below -20°C for optimum stability (it can be safely shipped at 2-8°C). The remainder of the kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

General Instructions

All reagents should be allowed to reach room temperature (18-25°C) before use.

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 (1) volume of the 10x stock with nine (9) 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

(PLEASE READ ATTACHED MSDS ON PAGE 4 FOR BIOHAZARD INFORMATION)

The monkey CRP standard is comprised of lyophilized Cynomolgus monkey serum of known CRP concentration. The CRP content was determined by reference to purified Cynomolgus monkey CRP prepared at Life Diagnostics Inc.

1. Reconstitute the lyophilized monkey CRP reference standard to a concentration of 2 $\mu\text{g/ml}$ by adding the volume of deionized or distilled water indicated on the vial label.
2. Label 8 polypropylene tubes as 75, 37.5, 18.75, 9.38, 4.69, 2.34, 1.17 and 0 ng/ml.
3. Into the tube labeled 75 ng/ml, pipette the volume of 1x diluent detailed on the CRP reference standard vial label. Then add the indicated volume of CRP standard (shown on the vial label) and mix gently. This provides the 75 ng/ml standard.
4. Dispense 250 μl of 1x diluent into the tubes labeled 37.5, 18.75, 9.38, 4.69, 2.34, 1.17 and 0 ng/ml.
5. Pipette 250 μl of the 75 ng/ml CRP standard into the tube labeled 37.5 ng/ml and mix. This provides the working 37.5 ng/ml CRP standard.

- Prepare an 18.75 ng/ml standard by diluting and mixing 250 μ l of the 37.5 ng/ml standard with 250 μ l of diluent in the tube labeled 18.75 ng/ml. Similarly prepare the 9.38, 4.69, 2.34, 1.17 ng/ml standards by serial dilution.

Please Note: The unused reconstituted reference standard should be aliquoted and stored frozen at or below -20°C (within 1 hour of reconstitution) if future use is intended.

SAMPLE PREPARATION

General Note: We find that CRP is present in normal pooled monkey serum at a concentration of ~5 μ g/ml and in-house studies indicate that acute phase concentrations can exceed 150 μ g/ml. We suggest that samples initially be diluted 1000 fold using the following procedure for each sample to be tested:

- Dispense 999 μ l of 1x diluent into one tube for each sample to be tested.
- Pipette 1.0 μ l of the serum sample into the tube containing 999 μ l of 1x diluent using a precision micro pipettor and mix. This provides a 1000 fold diluted sample.
- Repeat this procedure for each sample to be tested

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 μ l of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
- Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
- Remove the incubation mixture by flicking the plate contents into an appropriate Bio-waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
- Add 100 μ l of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 45 minutes.
- Wash as detailed in 4 to 5 above.
- Strike the wells sharply onto absorbent paper or paper towels to remove residual wash solution.
- Dispense 100 μ l of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μ l of Stop Solution to each well.
- Gently mix. It is important to make sure that all the blue color changes to yellow.
- Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

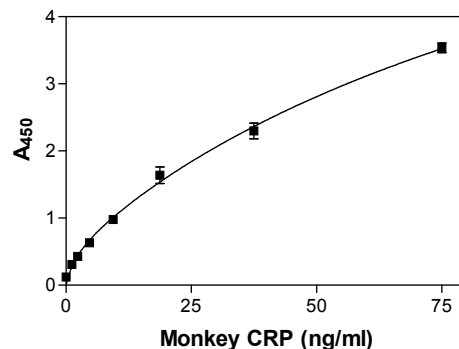
- Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of CRP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of CRP in the serum sample.
- If available, PC graphing software may be used for the above steps. We find that data usually fit well to a two site binding (hyperbola) equation.
- If the OD_{450} values of samples fall outside, or at the extremes, of the standard curve when tested at a dilution of 1000, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against CRP concentrations on the X axis is shown below. This curve is for the purpose of 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
0	0.122

Typical Monkey CRP Standard Curve



WARNINGS AND PRECAUTIONS

1. **CAUTION:** The lyophilized CRP standard contained in this kit was derived from *Cynomolgus* monkey serum. Please refer to the attached MSDS (page 4) prior to using the kit.
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Do not pipette reagents by mouth
5. **FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**

REFERENCES

1. Jinbo T, Hayashi S, Iguchi K, Shimizu 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, Suzuki Y, Kobune F, Ro S, Naiki M, Iguchi K, Yamamoto S. Concentrations of c-reactive protein in normal monkeys (*Macaca irus*) and in monkeys inoculated with *Bordetella 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, Vaheiri A and Lundkvist A. Wild-type Puumala Hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in *Cynomolgus* Macaques. *J. Virology.* 76:444-449 (2002)

MATERIAL SAFETY DATA SHEET

PRODUCT: Monkey CRP Standard (component of kit 2210-4)

CUSTOMER INFORMATION

Please forward a copy of this abbreviated MSDS to your coordinator for review and filing. Please assure that this MSDS reaches the intended user of this material.

HAZARD INFORMATION

HANDLE THIS MATERIAL AND ITS DERIVATIVES AS A BIOHAZARD

Nonhuman primates can carry a variety of zoonotic diseases including B virus (*Cercopithecine Herpes Virus 1* or *Herpesvirus simae*), Measles, Influenza, Pox viruses (Monkeypox and Yaba virus), filoviruses such as Ebola virus, Gastrointestinal disease (*Salmonella*, *Shigella*, *Giardia*, *Entamoeba histolytica*, *Balantidium coli*), Bacterial pneumonia (*Streptococcus pneumoniae*), and Tuberculosis (*Mycobacterium tuberculosis*). Zoonotic diseases are those that can be transmitted between species. It is important to note that a disease that does not cause serious health effects in one species may cause severe, life-threatening illness in another species.

Care must be taken by all personnel who handle this material to prevent potential exposure to zoonotic pathogens. Contact with this material may irritate the eyes, skin, or mucous membranes and potentially result in infection. In order to limit exposure, exercise all due caution and wear appropriate personal protective equipment when handling this material. Good laboratory and manufacturing procedures are essential for safe use. If eye exposure occurs, flush product from eyes with water for at least 15 minutes, see a physician. If skin exposure occurs, wash and scrub the exposed area thoroughly with soap, concentrated solution of detergent, povidone-iodine, or chlorhexidine and water, irrigate the area with running water for 15-20 minutes, see a physician.

FIRE AND SPILL INFORMATION

In case of fire use suitable extinguishing agent such as water, carbon dioxide, foam or dry chemical to suppress the surrounding fire. In case of spill collect material in a leak proof container and decontaminate the spilled material with a freshly made 1% bleach solution (a 1:5 dilution of household bleach) or similar disinfectant with virucidal properties, and dispose of according to Federal, State, and local regulations. Decontaminate the area of the spill with a freshly made 1% bleach solution (a 1:5 dilution of commercial bleach) or similar disinfectant with virucidal properties. Allow sufficient contact time (30 minutes) before final clean up of surfaces.

PERSONAL PROTECTIVE EQUIPMENT

Protective gloves, safety goggles, face shield, long sleeved lab coat or gown and access to a safety

eyewash station are recommended. Protective clothing should be replaced if it is contaminated. Protective clothing should be removed on leaving the work area. Wash hands after removing gloves.

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