KAMIYA BIOMEDICAL COMPANY

Monkey High-Sensitive CRP ELISA

For the quantitative determination of C-Reactive Protein (CRP) in monkey serum.

Cat. No. KT-425

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Monkey High-Sensitive CRP ELISA is an enzyme immunoassay for the quantitative determination of C-Reactive Protein (CRP) in monkey serum. For research use only.

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 µg/mL and levels may increase one hundred fold or more during the acute phase response.

PRINCIPLE
The ***K-ASSAY®*** Monkey High-Sensitive 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.

COMPONENTS
- Anti-monkey CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Monkey CRP Calibrator, lyophilized
- Diluent (10X), 25 mL
- Wash Solution (20X), 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 de-ionized water
- Polypropylene tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) before use.

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 de-ionized water.

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 de-ionized water.

CALIBRATOR PREPARATION

The monkey CRP calibrator is comprised of lyophilized serum of known CRP concentration. The CRP content was determined by reference to purified Cynomolgus monkey CRP prepared at Kamiya Biomedical Company.

1. Reconstitute the lyophilized monkey High-Sensitive CRP reference calibrator to a concentration of 2,000 ng/mL by adding 451.7 µL of de-ionized or distilled water.
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 481.25 µL of 1X diluent. Then add 18.75 µL of CRP calibrator and mix gently. This provides the 75 ng/mL calibrator.
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 calibrator into the tube labeled 37.5 ng/mL and mix. This provides the working 37.5 ng/mL CRP calibrator.
6. Prepare the 18.75 ng/mL calibrator by diluting and mixing 250 µL of the 37.5 ng/mL calibrator with 250 µL of diluent in the tube labeled 18.75 ng/mL. Similarly prepare the 9.38, 4.69, 2.34, 1.17 ng/mL calibrators by serial dilution.

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

SAMPLE PREPARATION

General Note: We find that CRP is present in normal pooled monkey serum at a concentration of ~5 µg/mL and in-house studies indicate that acute phase concentrations can exceed 150 µg/mL. We <u>suggest</u> that samples initially be diluted 1,000 fold using the following procedure for each sample to be tested:

1. Dispense 999 µL of 1X diluent into one tube for each sample to be tested.
2. Pipette 1.0 µL of the serum sample into the tube containing 999 µL of 1X diluent using a precision micro pipettor and mix. This provides a 1,000 fold diluted sample.
3. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

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

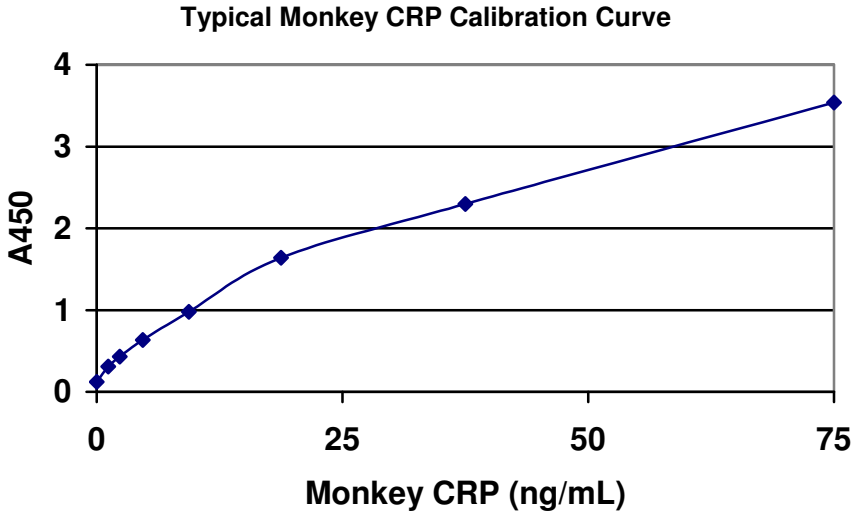
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of CRP in the serum sample.
5. 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.
6. If the A_{450} values of samples fall outside, or at the extremes, of the calibration curve when tested at a dilution of 1,000, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against CRP concentration 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 calibration 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



STORAGE

The lyophilized reference Calibrator should be stored at or below -20℃ for optimum stability. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

WARNINGS AND PRECAUTIONS

1. The calibrator used in this kit may contain human serum components. Any human material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Reagents in this kit and monkey samples must be handled according to the OHSA Standard on Bloodborne Pathogens or other appropriate national biohazard safety guidelines or regulations.
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.

FOR RESEARCH USE ONLY

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