KAMIYA BIOMEDICAL COMPANY

Sheep C-Reactive Protein (CRP) ELISA

For the quantitative determination of CRP in sheep serum and plasma

Cat. No. KT-786

For Research Use Only.

PRODUCT INFORMATION

Sheep C-Reactive Protein (CRP) ELISA
Cat. No. KT-786

PRODUCT
The ***K-ASSAY***® Sheep C-Reactive Protein (CRP) ELISA is an enzyme immunoassay for the quantitative determination of CRP in sheep serum and plasma. For research use only.

INTRODUCTION
CRP is an acute phase protein that is elevated in serum from most mammals because of infection and disease and can be used as a biomarker to evaluate health status.

PRINCIPLE
The assay uses affinity purified sheep CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated sheep CRP antibodies for detection. Calibrators 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 and 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, and absorbance is measured at 450 nm. The concentration of CRP is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- CRP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- CRP Calibrator
- 10X Diluent, 25 mL
- 20X Wash Solution, 50 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized 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 kit should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date if 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 procedure is carried out with a complete understanding of the instructions and 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℃. 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 de-ionized 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 de-ionized water.

CALIBRATOR PREPARATION

The CRP stock is comprised of purified sheep CRP lyophilized in a stabilizing matrix.

1. Reconstitute the lyophilized calibrator stock with 1.0 mL of H_2O. The reconstituted stock is stable for one day at 4℃ but should be aliquoted and frozen ≤ -20℃ if future use is intended.
2. Label 6 polypropylene or glass tubes: 250, 125, 62.5, 31.25, 15.63 and 7.81 ng/mL.
3. Prepare a 250 ng/mL working CRP calibrator by mixing 374.57 µL of diluent and 125.43 µL of reconstituted stock in the tube labeled 250 ng/mL.
4. Dispense 250 µL of diluent into the tubes labeled 125, 62.5, 31.25, 15.63 and 7.81 ng/mL.
5. Prepare a 125 ng/mL calibrator by diluting and mixing 250 µL of the 250 ng/mL calibrator with 250 µL of diluent in the tube labeled 125 ng/mL.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

SAMPLE PREPARATION

In studies, we found CRP levels ranging of approximately 90 µg/mL in normal sheep serum. To obtain values within the range of the calibration curve we suggest that samples be diluted 2,000 fold initially using the following procedure for each sample:

1. Dispense 98 µL and 292.5 µL of 1X diluent into two separate tubes.
2. Pipette and mix 2.0 µL of the serum/plasma sample into the first tube. This provides a 50 fold diluted sample.
3. Mix 7.5 µL of the 50-fold diluted sample with the 292.5 µL of diluent in the second tube. This provides a 2,000-fold dilution of the sample.

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℃ for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25℃ 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 adsorbent 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℃ for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto adsorbent 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 and 25℃ 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

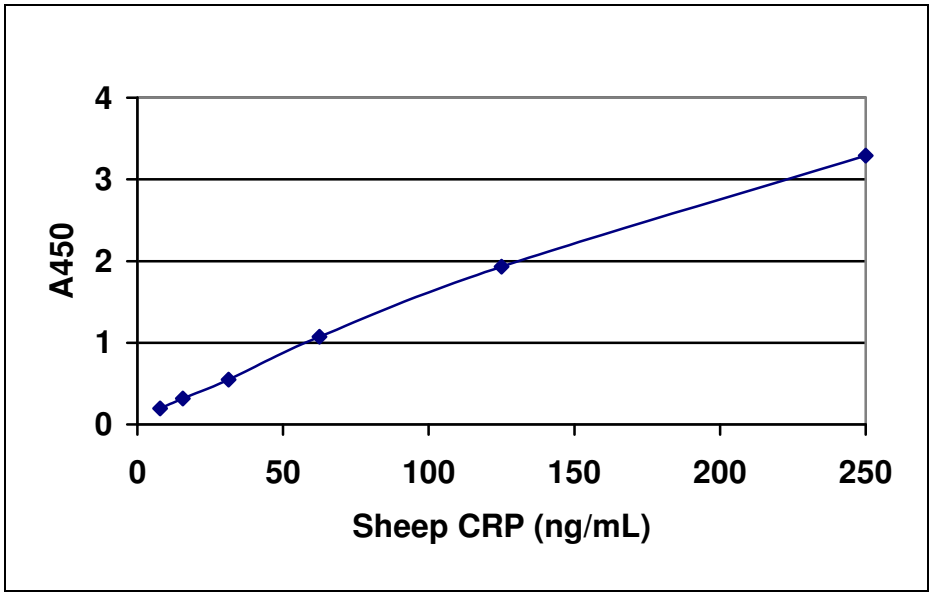
1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.

2. Fit the calibration curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration 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 calibration curve in each experiment.

CRP (ng/mL)	Absorbance (450 nm)
250	3.290
125	1.931
62.5	1.073
31.25	0.547
15.63	0.319
7.81	0.194



FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com