KAMIYA BIOMEDICAL COMPANY

Bovine C-Reactive Protein (CRP) ELISA

For the quantitative determination of CRP in bovine serum, plasma and milk

Cat. No. KT-784

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
CRP is an acute phase protein that is elevated in bovine serum and milk as a result of infection and disease and can be used as a biomarker to evaluate health status. Studies demonstrated that normal milk levels of CRP are 3.1±2.8 ng/mL (mean±SD, n=17). Levels of 4218±2658 ng/mL (mean±SD, n=20) were found in milk from cows with mastitis.

In contrast to bovine CRP ELISA kits from other vendors, this ELISA uses antibodies generated against bovine CRP.

PRINCIPLE
The ***K-ASSAY***® Bovine C-Reactive Protein (CRP) ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-bovine CRP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-bovine CRP antibodies for detection. Diluted samples and calibrators are first incubated in the microtiter wells for 45 minutes. The wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. CRP molecules are thereby sandwiched between the immobilization and detection antibodies. The wells are washed to remove unbound HRP-labeled antibodies. TMB Reagent is then added and incubated for 20 minutes. This results in the development of a blue color if CRP is present in the sample. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Absorbance is measured spectrophotometrically at 450 nm. The concentration of CRP is proportional to the absorbance and is derived from a calibration curve.

COMPONENTS
- Anti-bovine CRP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 mL
- Bovine CRP Stock
- 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 de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength with an optical density range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE
The unused kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit will remain stable until the expiration date provided that the components are stored as described above.

GENERAL INSTRUCTIONS
All reagents used directly in the assay should be allowed to reach room temperature (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 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
1. Reconstitute the lyophilized bovine CRP calibrator stock vial as described on the vial label. The reconstituted stock is stable for one day at 4°C but should be aliquoted and frozen at -20°C or lower if future use is intended.
2. Label 8 polypropylene or glass tubes as 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98 and 0 ng/mL.
3. Prepare a 62.5 ng/mL working CRP calibrator as detailed on the calibrator vial label by mixing the indicated volume of diluent and reconstituted stock in the tube labeled 62.5 ng/mL.
4. Dispense 250 µL of diluent into the tubes labeled 31.25, 15.63, 7.81, 3.91, 1.95, 0.98 and 0 ng/mL.
5. Prepare a 31.25 ng/mL calibrator by diluting and mixing 250 µL of the 62.5 ng/mL calibrator with 250 µL of diluent in the tube labeled 31.25 ng/mL. Similarly prepare the 15.63, 7.81, 3.91, 1.95 and 0.98 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION
Serum and Plasma: In studies we found CRP levels ranging from 15 - 165 µg/mL in bovine serum. In order to obtain values within the range of the calibration curve we suggest the serum and plasma samples be diluted 10,000 fold initially.
Milk: We found CRP levels of approximately 3 ng/mL in normal milk samples and measured samples after a 4-fold dilution. In milk from cows with mastitis we found levels ranging from 29 to 31,358 ng/mL. Optimal dilutions of mastitis milk must therefore be determined empirically but we suggest initially testing each mastitis milk sample at dilutions of 10, 50, 250 and 1,250 fold.

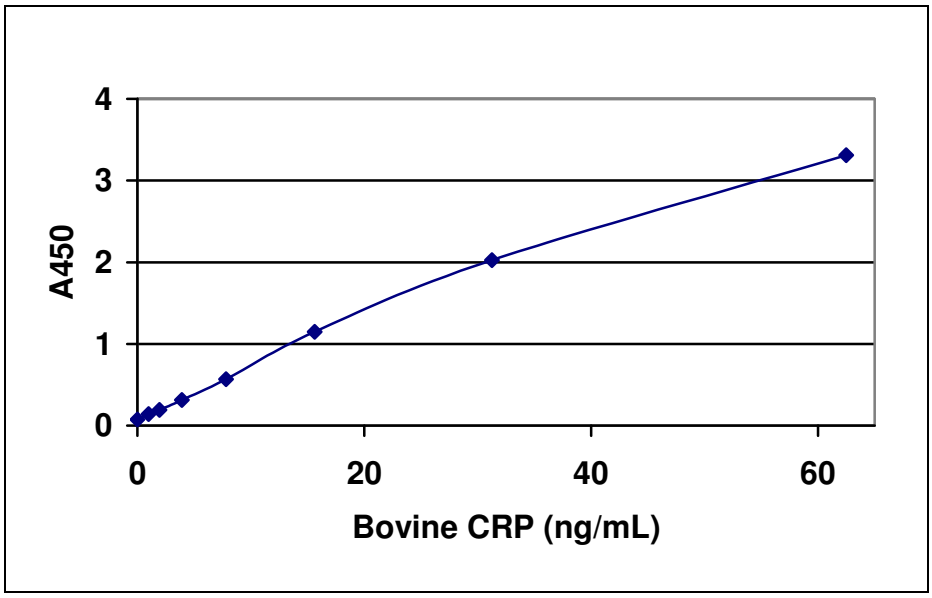
ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that calibrators and samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1X wash solution using a plate washer (400 µL/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 and 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. 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 concentrations 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/plasma sample.
5. If available, PC graphing software should be used for the above steps. We recommend a second order polynomial fit for the calibration curve.
6. If the OD_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A representative calibration curve with optical density readings at 450 nm 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 calibration curve in each experiment.

CRP (ng/mL)	Absorbance (450 nm)
62.5	3.309
31.25	2.026
15.63	1.147
7.81	0.569
3.91	0.314
1.95	0.191
0.98	0.140
0	0.073



LIMITATIONS OF THE PROCEDURE

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12784 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com