KAMIYA BIOMEDICAL COMPANY

Camel Serum Amyloid A (SAA) ELISA

For the quantitative determination of serum amyloid A (SAA) in camel serum

Cat. No. KT-779

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Camel Serum Amyloid A (SAA) ELISA is an enzyme immunoassay for the quantitative determination of serum amyloid A (SAA) in camel serum. For research use only.

INTRODUCTION
Serum amyloid A (SAA) is a positive acute phase protein of ~12 kDa that is expressed in the liver and circulates in blood. It is a useful biomarker of inflammation and disease in many species including camelids. This assay recognizes SAA in camel and alpaca serum. Reactivity with llama SAA has not yet been investigated.

PRINCIPLE
The **K-ASSAY®** Camel Serum Amyloid A (SAA) ELISA uses two peptide-specific antibodies that recognize different epitopes on camel SAA 2. One is used for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), is used for detection. Serum samples are diluted at least 4-fold and 100 µL aliquots are then incubated in the antibody-coated microtiter wells together with HRP conjugate (100 µL) for two hours. SAA molecules, if present, are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB Reagent is added and incubated for 30 minutes. This results in the development of a blue color if SAA is present. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured at 450 nm. The concentration of SAA is proportional to the optical density and is derived from a calibration curve.

COMPONENTS
- Anti-camel SAA coated 96-well microtiter (12 x 8 wells)
- HRP Conjugate, 11 mL
- SAA stock, 1 vial (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB Reagent, 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
- 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 (25℃) before use.
Please take the time to completely read and understand this kit insert before starting your assay. Don't hesitate to contact us by telephone or e-mail should you require technical assistance or clarification.

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

The diluent is specially formulated to allow SAA measurement in camelid serum. It is provided ready to use. Do not substitute other buffers. A slight precipitate may form during storage. It can be removed by centrifugation for 5 minutes at 3,000 rpm.

SAMPLE PREPARATION

Studies indicate that camelid serum should be diluted at least 4-fold. Plasma should not be used unless diluted 50-fold or greater. Optimal dilution should be determined by the researcher.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock as described on the vial label. Mix gently several times before use.
2. Label 8 polypropylene tubes as 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 0 ng/mL.
3. Into the tube labeled 500 ng/mL, pipette the volume of diluent detailed on the SAA stock vial label. Then add the volume of SAA stock indicated on the vial label and mix gently. This provides the working 500 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 250, 125, 62.5, 31.25, 15.63, 7.81 and 0 ng/mL.
5. Pipette 250 µL of the 500 ng/mL SAA calibrator into the tube labeled 250 ng/mL and mix. This provides the working 250 ng/mL SAA calibrator.
6. Similarly prepare the 125, 62.5, 31.25, 15.63, 7.81 ng/mL calibrators by serial dilution.

Please Note: Unused reconstituted reference calibrator stock should be stored frozen at or below -20°C if future used is intended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be tested in duplicate).
3. Add 100 µL of HRP conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for two hours.
5. Wash and empty the microtiter wells 6 times with 1X wash solution using a plate washer (400 µL/well). 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. Dispense 100 µL of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 30 minutes.
9. Stop the reaction by adding 100 µL of Stop Solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

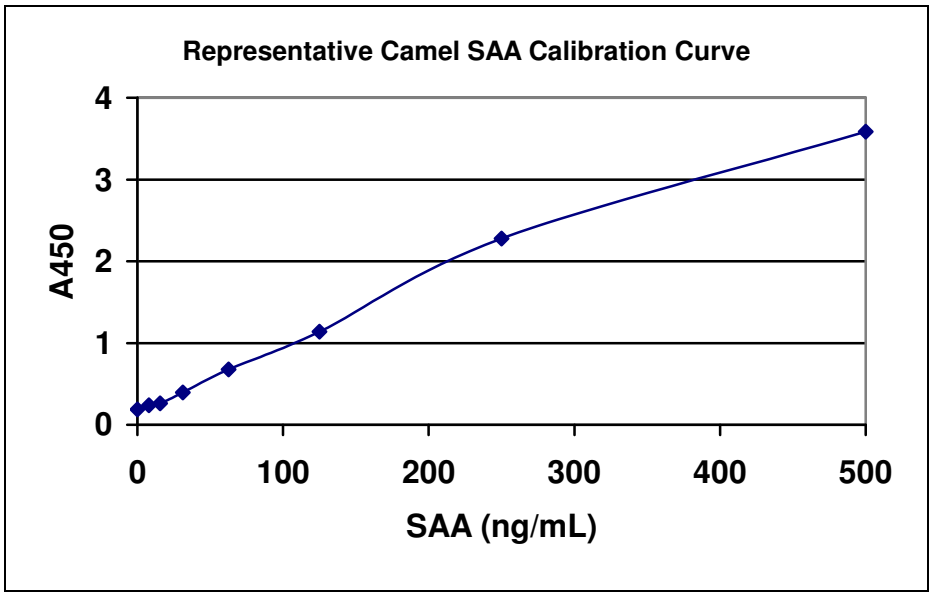
CALCULATION OF RESULTS

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 SAA in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of SAA in the serum sample.
5. If available, PC graphing software should be used for the above steps. We find that a good fit of the data is obtained with either a two site binding equation or a second order polynomial equation.
6. If the A_{450} values of samples fall outside the range of the calibration curve samples should be re-diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A representative calibration curve with optical density readings at 450 nm on the Y-axis against SAA 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.

SAA (ng/mL)	Absorbance (450 nm)
500	3.584
250	2.278
125	1.137
62.5	0.677
31.25	0.395
15.63	0.263
7.81	0.238
0	0.189



STORAGE

Upon receiving the kit please store the SAA calibrator in a freezer at or below -20℃. The remaining components of the kit should be stored in a refrigerator at 4℃. It is important that 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.

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