KAMIYA BIOMEDICAL COMPANY

Dolphin Serum Amyloid A (SAA) ELISA

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

Cat. No. KT-790

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Dolphin Serum Amyloid A (SAA) ELISA is an enzyme immunoassay for the quantitative determination of serum amyloid A (SAA) in dolphin 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. In studies we found SAA levels of 471 ± 113 ng/mL (mean±SE, n=6) in serum from healthy dolphins. In serum from dolphins identified as sick, we found SAA levels of 3,268 ± 2,471 ng/mL (mean±SE, n=7). These findings suggest that SAA is a positive acute phase protein in dolphins also.

PRINCIPLE
The ***K-ASSAY®*** Dolphin Serum Amyloid A (SAA) ELISA uses two peptide-specific antibodies that recognize different epitopes on dolphin (Tursiops truncates) 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 50-fold and 100 µL aliquots are then incubated in the antibody-coated microtiter wells together with HRP conjugate (100 µL) for one hour. SAA molecules, if present, are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB Reagent is added and incubated for 20 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. 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-dolphin 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

Note: The SAA calibrator consists of a synthetic dolphin SAA polypeptide that encompasses the epitopes recognized by the antibodies used in this kit. The concentration stated on the vial refers to the equivalent concentration of full length dolphin SAA. The synthetic peptide and native SAA are recognized identically.

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°C) 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 dolphin 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 dolphin serum should be diluted at least 50-fold. 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 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 ng/mL.
3. Into the tube labeled 50 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 50 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.125, 1.563 and 0.781 ng/mL.
5. Pipette 250 µL of the 50 ng/mL SAA calibrator into the tube labeled 25 ng/mL and mix. This provides the working 25 ng/mL SAA calibrator.
6. Similarly prepare the remaining 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 one hour.
5. Wash and empty the microtiter 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.
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 20 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

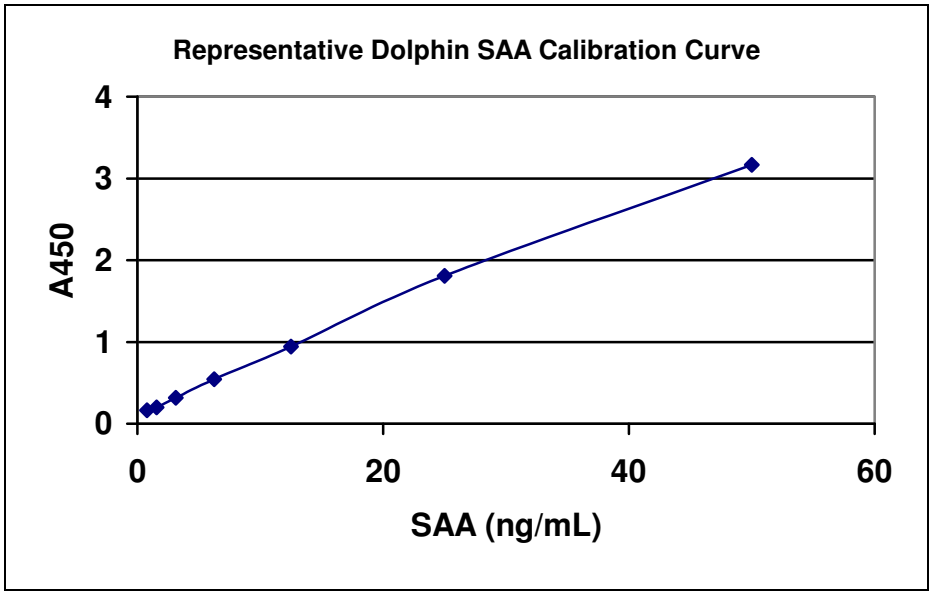
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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)
50	3.167
25	1.807
12.5	0.945
6.25	0.543
3.125	0.317
1.563	0.198
0.781	0.164



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

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