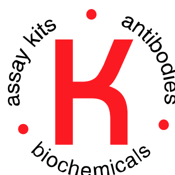

KAMIYA BIOMEDICAL COMPANY

Pig Serum Amyloid A (SAA) ELISA

For the quantitative determination of serum amyloid A (SAA) in pig serum or plasma

Pig. No. KT-748

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
SAA, a protein of ~12 kDa, is a positive acute phase reactant that circulates in blood mostly associated with high density lipoprotein (HDL). In pigs, basal serum levels are ~5 µg/mL but levels can reach 500 µg/mL as a result of infection. SAA is considered to be an excellent acute phase biomarker in pigs.

PRINCIPLE
The ***K-ASSAY®*** Pig Serum Amyloid A ELISA uses two peptide-specific antibodies that recognize different epitopes. One is used for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), is used for detection. Serum or plasma samples are first incubated at 60℃ for one hour in order to dissociate SAA from HDL. Following heat treatment, the samples are diluted at least 400-fold. The diluted samples (100 µL) are then incubated in the antibody-coated microtiter wells together with HRP conjugate (100 µL) for one hour at 25℃. As a result, SAA molecules 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 20 minutes at 25℃. 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-pig SAA coated with 96-well microtiter (12 x 8 wells)
- HRP Conjugate, 11 mL
- SAA stock, 3 vials (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 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
- 60℃ water bath or incubator
- 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.

SAMPLE PREPARATION

Heat treatment

1. Dispense 100 µL of serum or plasma into a polypropylene microcentrifuge tube and seal the cap.
2. Repeat this procedure for each sample to be tested.
3. Incubate the samples in a water bath or incubator pre-equilibrated to 60°C for exactly one hour (after heat treatment, the samples can be stored in the refrigerator for at least 3 days).

Dilution

After heat treatment, the sample should be diluted at least 400-fold in diluent. Optimum dilution factors must be determined empirically.

CALIBRATOR PREPARATION

1. Reconstitute the SAA stock as described on the vial label. Mix gently several times before use. The stock does not require heat treatment. (**The reconstituted calibrator should be aliquoted and frozen at or below -20°C after reconstitution if use beyond this time is intended).**
2. Label 7 polypropylene tubes as 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/mL.
3. Into the tube labeled 100 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 100 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/mL.
5. Pipette 250 µL of the 100 ng/mL SAA calibrator into the tube labeled 50 ng/mL and mix. This provides the working 50 ng/mL SAA calibrator.
6. Similarly prepare the remaining calibrators by serial dilution.

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

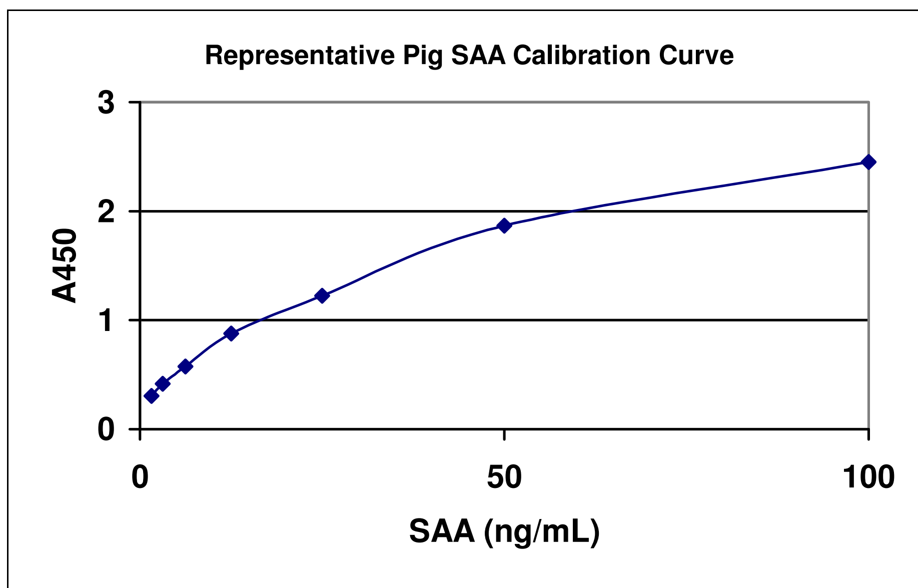
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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)
100	2.451
50	1.867
25	1.224
12.5	0.877
6.25	0.576
3.125	0.416
1.563	0.305



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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