KAMIYA BIOMEDICAL COMPANY

Salmon Serum Amyloid A (SAA) ELISA

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

Cat. No. KT-778

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
SAA, a protein of ~12 kDa, is a positive acute phase reactant expressed primarily in the liver that circulates in blood. In salmon, SAA gene expression increases >5 fold during sea lice infection. At the time of writing, SAA protein levels in plasma or serum from normal and sick salmon had not been reported. This kit allows measurement of SAA protein levels in salmon plasma.

PRINCIPLE
The ***K-ASSAY***® Salmon Serum Amyloid A (SAA) ELISA uses two peptide-specific antibodies that recognize different epitopes on salmon SAA. One is used for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), is used for detection. Serum samples are first incubated at 60℃ for one hour to dissociate SAA from lipoproteins. Following heat treatment, the samples are centrifuged and the supernatants diluted at least 20-fold. The diluted samples (100 µL) are then incubated in the antibody-coated microtiter wells together with HRP conjugate (100 µL) for one hour. 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. 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-salmon 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 microfuge tubes (1.5 mL)
- 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)
- 60℃ water bath or incubator

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 0.5 mL of plasma into a 1.5 mL polypropylene micro centrifuge 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℃ for exactly one hour.
4. Cool the samples by placing the tubes in a bath of water at room temperature for 5 minutes.
5. After heating the plasma sample will likely form a viscous gel. Vortex or flick the bottom of the microfuge tube to mix the sample. Grasping the tube tightly by the cap and flicking the bottom of the tube with a pen works well.
6. Centrifuge the samples in a microfuge at 14,000 rpm for 15 minutes.
7. Aspirate and save the supernatant.

Dilution

After heat treatment, the sample should be diluted at least 20-fold in diluent.

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.
2. Label 8 polypropylene tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 and 0 ng/mL.
3. Into the tube labeled 2.5 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 2.5 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 and 0 ng/mL.
5. Pipette 250 µL of the 2.5 ng/mL SAA calibrator into the tube labeled 1.25 ng/mL and mix. This provides the working 1.25 ng/mL SAA calibrator.
6. Similarly prepare the 0.625, 0.313, 0.156, 0.078, 0.039 ng/mL calibrators by serial dilution.

Please Note: Unused reconstituted reference calibrator stock should be stored frozen at or below -20℃ 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℃) 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℃) 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

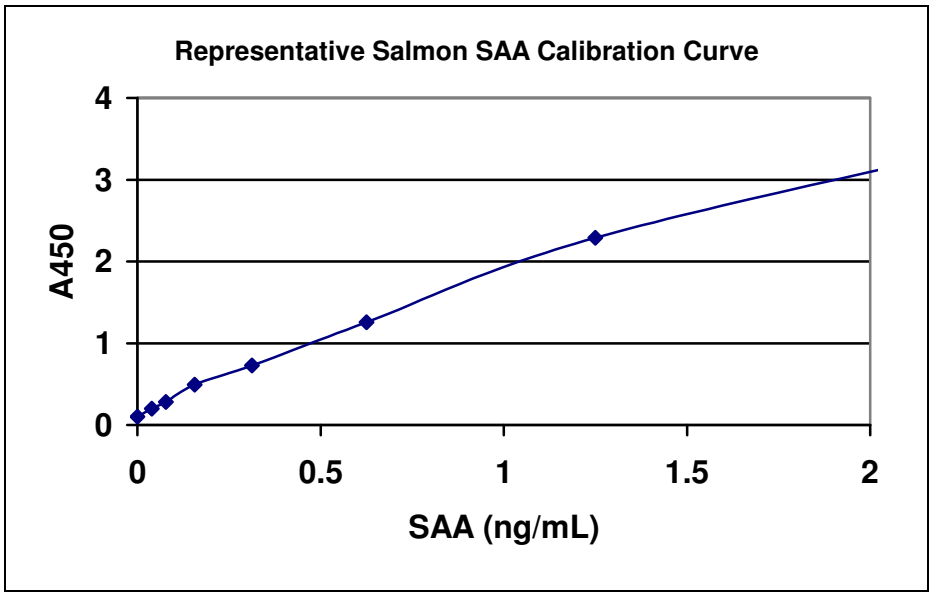
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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)
2.5	3.604
1.25	2.288
0.625	1.258
0.313	0.728
0.156	0.495
0.078	0.282
0.039	0.201
0	0.102



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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