# SALMON SERUM AMYLOID A (SAA) ELISA
# Life Diagnostics, Inc., Catalog Number: SAA-19

## INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of ≈12 kDa that is expressed in lever and circulates in blood). In salmon, SAA gene expression increases five-fold during sea lice infection.[1] 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 OF THE ASSAY

The assay uses two different peptide-specific salmon SAA antibodies; one for solid phase immobilization (microtiter wells) and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum samples are first incubated at 60°C for one hour to dissociate SAA from lipoproteins. After heat treatment, the samples are centrifuged. The supernatants are diluted at least 20-fold. Standards and diluted samples are incubated in the microtiter wells together with HRP conjugate for one hour. This results in SAA molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If SAA is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of SAA is proportional to absorbance and is derived from a standard curve.

## MATERIALS AND COMPONENTS

*Materials provided with the kit:*

- SAA antibody coated 96-well plate (12 x 8-well strips)
- HRP conjugate, 11 ml
- SAA stock (lyophilized)[a]
- 20x Wash solution; TBS50-20, 50 ml
- Diluent; YD50-1, 50 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

*Materials required but not provided:*

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Water bath
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

## STORAGE

The SAA stock should be stored at or below -20°C. The remainder of the kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

## GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

## 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 deionized water.

## STANDARD 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 standard.
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 standard into the tube labeled 1.25 ng/ml and mix. This provides the 1.25 ng/ml SAA standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Unused stock should be stored frozen at or below -20°C if future use is intended.

## SAMPLE PREPARATION

Heat treatment[b]

1. Dispense 0.5 ml of each plasma sample into a 1.5 ml polypropylene micro centrifuge tube and seal the cap.
2. Incubate the samples in a water bath or incubator pre-equilibrated to 60°C for exactly one hour.
3. Cool the samples by placing the tubes in a bath of water at room temp for 5 minutes.
4. 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.
5. Centrifuge the samples in a microfuge at 14,000 rpm for 15 minutes.
6. Aspirate and save the supernatant.

[a] The SAA standard consists of a synthetic salmon 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 salmon SAA. The synthetic peptide and native SAA are recognized identically.

[b] We find that heat treatment significantly improves SAA recovery.

### Dilution

After heat treatment, the sample should be diluted at least 20-fold with diluent to avoid matrix effects. Optimal dilutions must be determined empirically.

## ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 2-8°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Add 100 µl of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µl of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20-minutes, 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 absorbance at 450 nm with a plate reader within 5 minutes.

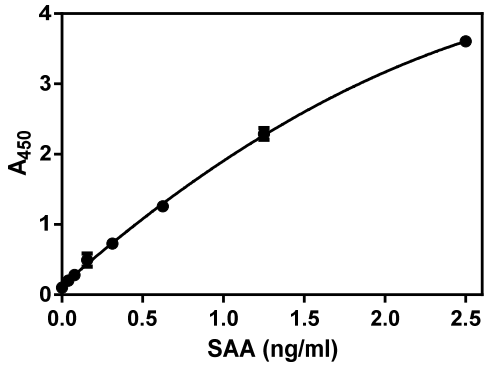
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum sample.
4. If the $A_{450}$ values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

## TYPICAL STANDARD CURVE

A typical standard curve with absorbance at 450 nm on the Y-axis against SAA concentrations on the X-axis is shown below. This curve is for illustration only.

| SAA (ng/ml) | $A_{450}$ |
|---|---|
| 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 |




## REFERENCES

1. Sutherland BJG, et al. Comparative transcriptomics of Atlantic Salmo salar, chum Oncorhynchus keta and pink salmon O. gorbuscha during infections with salmon lice Lepeophtheirus salmonis. BMC Genomics. 15:200 (2014). http://www.biomedcentral.com/1471-2164/15/200

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