

DOLPHIN SERUM AMYLOID A (SAA) ELISA

Life Diagnostics, Inc., Catalog Number: SAA-18

INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of ≈ 12 kDa that is expressed in liver and circulates in blood. It serves as a useful biomarker of inflammation and general health in dolphins.¹

PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific dolphin SAA antibodies; one for solid phase immobilization (microtiter wells) and the other, conjugated to horseradish peroxidase (HRP), for detection. 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. 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: CSD50-1, 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 4°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 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 indicated volume of stock and mix gently. This provides the 50 ng/ml standard.
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 standard into the tube labeled 25 ng/ml and mix. This provides the 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

In studies at Life Diagnostics we found SAA levels of 471 ± 113 ng/ml (mean \pm SE, n=6) in serum from healthy dolphins. In serum from dolphins identified as sick, we found SAA levels of 3268 ± 2471 ng/ml (mean \pm SE, n=7). To avoid matrix effects serum should be diluted at least 50-fold prior to assay. Optimal dilution should be determined by the researcher.

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 4°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.

^a The SAA standard 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.

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.

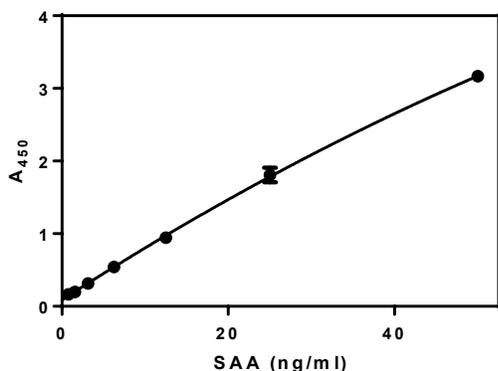
CALCULATION OF RESULTS

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}
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



REFERENCES

1. Miller SN. et al. Serum amyloid A in healthy female bottlenose dolphins (*tursiops truncatus*) during and after uncomplicated pregnancy. *Aquatic Mammals* 43(4):417-420 (2017)

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