

# PIG SERUM AMYLOID A (SAA) ELISA

## Life Diagnostics, Inc., Catalog Number: SAA-9

### INTRODUCTION

SAA, a protein of approximately 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 approximately 5 µg/ml.<sup>1</sup> Levels can reach 500 µg/ml during infection.<sup>2,3</sup>

### PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific pig SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum or plasma samples are first denatured by heating for 1 hour at 60°C. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured samples are diluted. 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 stock
- SAA stock (lyophilized)
- 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 and the HRP conjugate stock should be stored at or below -20°C for optimum stability. 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.

### DILUENT

The diluent is specially formulated for measurement of SAA in pig serum or plasma. It is provided ready to use. Do not substitute other buffers.

### HRP CONJUGATE PREPARATION

The anti-pig SAA HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock with the diluent provided with the kit as described on the stock vial label.

### 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, 3.13, 1.56 and 0.78 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 SAA 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, 3.13, 1.56 and 0.78 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

### Denaturation

1. Dispense 100  $\mu$ l of each serum or plasma sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

### Dilution

1. Dilute the denatured samples at least 400-fold with the diluent provided with the kit. Optimum dilutions must be empirically determined.

## 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  $\mu$ l of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Add 100  $\mu$ l 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  $\mu$ l/well).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100  $\mu$ l 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  $\mu$ l 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.

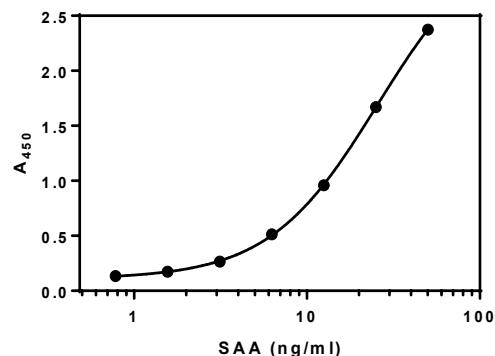
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus  $\log_{10}$  of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis =  $\log_{10}$  concentration) and determine the concentration of the samples from the standard curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma 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	2.374
25	1.670
12.5	0.959
6.25	0.514
3.13	0.266
1.56	0.174
0.78	0.134



## REFERENCES

1. Pomorska-Mol M, Kwit K and Markowska-Daniel I. Major acute phase proteins in pig serum from birth to slaughter. Bull Vet Inst Pulawy 56:553-557 (2012)
2. Hulten C, Johansson E, Fossum C and Wallgren P. Interleukin 6, serum amyloid A and haptoglobin as markers of treatment efficacy in pigs experimentally infected with Actinobacillus pleuropneumoniae. Veterinary Microbiology 95:75-79 (2003)
3. Pomorska-Mol M, Markowska-Daniel I and Kwit K. Acute phase protein response in pigs experimentally co-infected with swine influenza virus and Bordetella bronchiseptica. Centr. Eur. J. Immunol. 37(3):221-226 (2012)

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For technical assistance please email us at  
techsupport@lifediagnosics.com