COW SERUM AMYLOID A (SAA) ELISA
Life Diagnostics, Inc., Catalog Number: SAA-11

INTRODUCTION

SAA, a protein of approximately 12 kDa, is a positive acute phase reactant that circulates in blood. Serum levels are increased because of inflammation and infection. In serum from healthy cows we found levels of 0.16±0.22 μg/ml (mean±SD, n=10). In serum from sick cows, levels were 4.93±5.6 μg/ml (mean±SD, n=10).

PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific cow SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum 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, 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 stock
- SAA stock (lyophilized)
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: CSD50-1, 2 x 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 must 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.

DILUENT

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

HRP CONJUGATE PREPARATION

The anti-cow 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 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 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 indicated volume of stock and mix gently. This provides the 100 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Pipette 250 μl of the 100 ng/ml SAA standard into the tube labeled 50 ng/ml and mix. This provides the 50 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

Serum can be used with this kit. Plasma should not be used.

Denaturation

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

Dilution

After denaturation dilute each sample at least 25-fold with diluent. We found that most serum samples gave at least one value within range of the standard curve if tested at dilutions of 100, 200, 400 and 800-fold. Optimal dilutions must be determined empirically.

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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.
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.

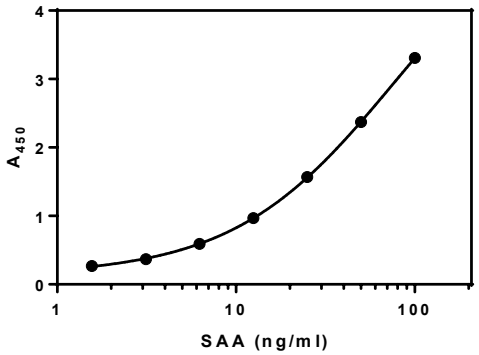
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ 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 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}
100	3.308
50	2.370
25	1.567
12.5	0.968
6.25	0.592
3.13	0.369
1.56	0.269



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