GOAT SERUM AMYLOID A ELISA
Life Diagnostics, Inc., Catalog Number: SAA-13

GOAT SAA ELISA

INTRODUCTION
Serum amyloid A (SAA) is a positive acute phase protein of ~12 kDa. In goats, amino acid sequences for three isoforms have been reported at the time of writing: A3, A4 and X2. The A3 and X2 isoforms are expressed in extra hepatic tissues including mammary tissue (ref 1). The A4 isoform is expressed in the liver.

PRINCIPLE OF THE ASSAY
The goat SAA ELISA uses two peptide-specific antibodies developed at Life Diagnostics, Inc. that recognize different epitopes present in goat SAA A3 and X2. One antibody is used for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), is used for detection. Serum samples are diluted and incubated in the microtiter wells together with the HRP conjugate for one hour. SAA molecules, if present, 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. Optical density is measured at 450 nm. The concentration of SAA is proportional to the optical density and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- Anti-goat SAA coated 96-well microtiter (12x8 wells)
- HRP conjugate stock, 50 μl
- SAA stock, 1 vial (lyophilized)1. Store at -20°C.
- 20x Wash Buffer, 50 ml
- Diluent (CSDT50-1), 50 ml
- TMB Reagent, 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:
- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker with an approximate mixing speed of 150 rpm
- Plate reader capable of measuring absorbance at 450 nm
- PC graphing software or graph paper

STORAGE
Upon receiving the kit, please store the SAA standard in a freezer at or below -20°C. The remaining components of the kit should be stored in a refrigerator at 2-8°C. It is important that the microtiter plate be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase, provided that the components are stored as described above.

1 The SAA standard consists of a synthetic goat 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 goat SAA A3

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature (25°C) before use.
2. Please take the time to completely read and understand this kit insert before starting your assay. Don't hesitate to contact Life Diagnostics by telephone or email 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 deionized water.

DILUENT
The diluent is formulated to allow SAA measurement in goat serum and milk. It is provided ready to use. Do not substitute other buffers.

SAMPLE PREPARATION
Samples should be diluted at least 10-fold with the diluent provided with the kit. Optimal dilutions must be determined empirically.

HRP CONJUGATE PREPARATION
The anti-goat 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.
2. Label 8 polypropylene tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 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 volume of SAA stock indicated on the vial label and mix gently. This provides the working 100 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 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 working 50 ng/ml SAA standard.
6. Similarly prepare the 25, 12.5, 6.25, 3.13 and 1.56 ng/ml standards by serial dilution.

Please Note: Unused reconstituted reference standard stock should be stored frozen at or below -20°C if future use is intended.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Add 100 μl of diluted HRP conjugate into each well.
4. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C)2 for one hour.

2 The ELISA was validated using a shaking incubator at 25°C and 150 rpm. Lower temperatures and/or mixing speeds may result in lower absorbance values.
5. Wash and empty the microtiter wells 6 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 absorbent 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°C) 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 standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard 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 standard 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 to either a single site binding equation or a second order polynomial equation.
6. If the A_{450} values of samples fall outside the range of the standard curve samples should be re-diluted appropriately and re-tested.

**TYPICAL STANDARD CURVE**

A representative standard curve with optical density readings at 450 nm on the Y-axis against SAA concentrations 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 standard curve in each experiment.

<table>
<thead>
<tr>
<th>SAA (ng/ml)</th>
<th>A_{450}</th>
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<tbody>
<tr>
<td>100</td>
<td>2.516</td>
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<tr>
<td>50</td>
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LDI RESULTS

We found SAA levels of 62±23 ng/ml (mean±SD, n = 16) in serum from healthy goats.

**REFERENCES**


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