MOUSE SERUM AMYLOID A (SAA) ELISA TEST KIT
Life Diagnostics, Inc., Catalog Number: SAA-1

Mouse SAA ELISA

INTRODUCTION
SAA is an acute phase serum protein that is elevated in mice approximately 50-fold following lipopolysaccharide injection. In mice two major forms of SAA are induced during the acute phase response, SAA1 and SAA2. Studies have shown that the two forms are similarly increased in response to different inflammatory stimuli. This ELISA kit uses antibodies that preferentially detect SAA2. Measurement of SAA provides a useful biomarker of inflammation and disease.

PRINCIPLE OF THE TEST
The mouse SAA test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified peptide-specific polyclonal anti-mouse SAA2 antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated polyclonal peptide-specific anti-mouse SAA1/2 antibodies for detection. The test sample is diluted and incubated in the microtiter wells together with the HRP conjugate for one hour. This results in SAA2 molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of SAA2 is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS
Materials provided with the kit:
- Anti-mouse SAA2 antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 ml
- Reference standard (0.20 ml, lyophilized), containing mouse SAA (concentration and dilution instructions are detailed on the vial label)
- 20x Wash Buffer, 50 ml
- Diluent, 30 ml
- TMB Reagent (One-Step), 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
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- Plate reader capable of measuring OD at 450 nm
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

The lyophilized reference standard should be stored at or below -20°C for optimum stability (it can be safely shipped at 2-8°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 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.

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum or plasma samples should generally be diluted ~100 fold or more with diluent in order to obtain values within the standard range.

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
The mouse SAA standard is comprised of lyophilized mouse serum of known SAA concentration. The SAA content was determined by reference to a synthetic mouse SAA2 polypeptide.

1. Reconstitute the lyophilized mouse SAA reference standard by addition of 200 μl of deionized or distilled water. Mix gently several times over a period of 5-10 minutes. The concentration of SAA in the reconstituted stock is indicated on the vial label.
2. Label 7 polypropylene tubes as 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 ng/ml.
3. Into the tube labeled 500 ng/ml, pipette the volume of diluent detailed on the SAA reference standard vial label. Then add the indicated volume of reference SAA standard and mix gently. This provides the working 500 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 250, 125, 62.5, 31.25, 15.6, and 7.8 ng/ml.
5. Pipette 250 μl of the 500 ng/ml SAA standard into the tube labeled 250 ng/ml and mix. This provides the working 250 ng/ml SAA standard.
6. Prepare a 125 ng/ml standard by diluting and mixing 250 μl of the 250 ng/ml standard with 250 μl of diluent in the tube labeled 125 ng/ml. Similarly prepare the 125, 62.5, 31.25, 15.6 and 7.8 ng/ml standards by serial dilution.

Please Note: The reconstituted reference standard should be aliquoted and stored frozen at or below -20°C (within 1 hour of reconstitution) if future use is intended.

SAMPLE PREPARATION
General Note: Because SAA levels can increase as much as 50 fold or more during inflammation, optimal dilutions should be determined empirically. However, as a good starting point, samples may be tested at a 100 fold dilution using the following procedure for each sample to be tested:

1. Dispense 297 μl of diluent into a polypropylene tube.

A Studies at Life Diagnostics, Inc. indicate that this kit has less than 5% cross reactivity with mouse SAA1 polypeptide compared to mouse SAA2 polypeptide.
2. Pipette and mix 3 µl of the serum sample into the tube containing 297 µl of diluent. This provides a 100 fold diluted sample.
3. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Add 100 µl of HRP conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for one hour.
5. Wash and empty the microtiter wells 6 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or a squirt bottle. 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 (18-25°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 (A₄₅₀) 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 good a good fit of the data is obtained to a two site binding equation.
6. If the OD₄₅₀ values of samples fall outside the standard curve when tested at a dilution of 100, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE
A typical standard curve with optical density readings at 450nm 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>SAA2 (ng/ml)</th>
<th>A₄₅₀</th>
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<tbody>
<tr>
<td>500</td>
<td>2.538</td>
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<tr>
<td>250</td>
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<tr>
<td>125</td>
<td>1.200</td>
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<tr>
<td>62.5</td>
<td>0.874</td>
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<tr>
<td>31.25</td>
<td>0.654</td>
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<td>15.6</td>
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<td>7.8</td>
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REFERENCES

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