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

## INTRODUCTION

SAA is an acute phase serum protein that is elevated in mice approximately 50-fold following lipopolysaccharide injection.[1] 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.[2]

## PRINCIPLE OF THE ASSAY

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. Standards and diluted samples are incubated, together with HRP conjugate, in the microtiter wells 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 the 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)
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: YD30-1, 30 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
- 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 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.

## STANDARD PREPARATION

The SAA stock is comprised of lyophilized mouse serum of known SAA concentration.

1. Reconstitute the stock with deionized or distilled water as described on the vial label. Mix gently several times over a period of 5-10 minutes.
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 stock vial label. Then add the indicated volume of reference SAA stock and mix. This provides 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. Prepare the 250 ng/ml standard by mixing 250 µl of the 500 ng/ml SAA with 250 µl of diluent in the tube labeled 250 ng/ml.
6. Prepare the remaining standards by two-fold serial dilution.

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

## SAMPLE PREPARATION

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 (mix 3 µl of sample with 297 µl of diluent).

## 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.

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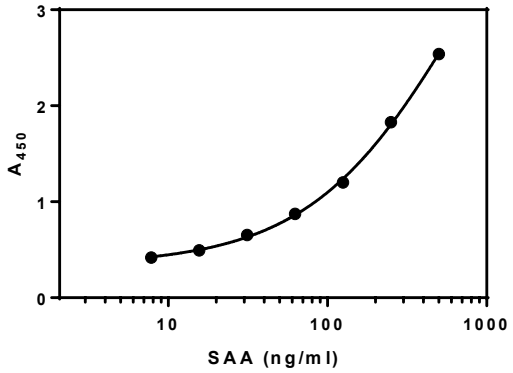
## CALCULATION OF RESULTS

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 concentration from 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) | $A_{450}$ |
|---|---|
| 500 | 2.538 |
| 250 | 1.828 |
| 125 | 1.200 |
| 62.5 | 0.874 |
| 31.25 | 0.654 |
| 15.6 | 0.493 |
| 7.8 | 0.419 |




## REFERENCES


1. Hoffman JS and Benditt EP. Changes in high density lipoprotein content following endotoxin administration in the mouse: Formation of serum amyloid protein-rich subfractions. J. Biol Chem. 257: 10510-10517 (1982)
2. Foyn Brun C, Sletten K and Marhaug G. Mouse serum amyloid A (SAA) proteins isolated by two-dimensional electrophoresis: characterization of isotypes and the effect of separate and combined administrations of cytokines, dexamethasone and lipopolysaccharide (LPS) on serum levels and isotype distribution. Clin Exp Immunol. 111: 231-236 (1988)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For technical assistance please email us at
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