INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein that is expressed in the liver and circulates in blood. In Rainbow Trout, whole-body transcriptomic studies have demonstrated that SAA is highly induced following bacterial challenge. In studies at Life Diagnostics, we found that SAA was significantly increased in serum and heart extracts from trout with bacterial infections.

PRINCIPLE OF THE ASSAY

The assay uses rabbit polyclonal trout SAA antibodies. It recognizes Rainbow Trout and Atlantic Salmon SAA. Unconjugated antibody is used for solid phase immobilization (microtiter wells). Horseradish peroxidase (HRP) conjugated antibody is used for detection. Standards and diluted serum or tissue extracts (100 µl) are pipetted into the antibody coated microtiter wells and incubated for 45 minutes. After washing the wells, HRP conjugate (100 µl) is added to the wells and incubated for 45 minutes. After a final wash, TMB (100 µl) 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

Materials provided with the kit:

- Anti-SAA coated plate (12 x 8-well strips)
- HRP conjugate, 12 ml.
- SAA stock, 1 vial. **Store ≤ -70°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: SB50-1, 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 tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Store the standard stock vial at or below -70°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. The kit 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 conducted with a complete understanding of the instructions and with adherence to good laboratory practice.
3. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate[1]. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µl aliquots to the wells of the antibody-coated plate.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

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. Unused wash buffer may be stored at 4°C for one week.

DILUENT

The diluent (SB50-1) is formulated for measurement of SAA in serum and tissue extracts. It is supplied ready to use. DO NOT substitute other buffers.

STANDARDS

1. The stock consists of 20 µg/ml recombinant trout SAA in a stabilizing buffer. It should be stored at or below -70°C and thawed shortly before use. Return unused stock to the freezer after use.
2. Prepare the 100 ng/ml standard from the stock by mixing 2.5 µl of stock with 497.5 µl of SB50-1 diluent.
3. Label seven polypropylene tubes as 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/ml. Dispense 0.25 ml of diluent into each.
4. Pipette 0.25 ml of the 100 ng/ml SAA standard into the tube labeled 50 ng/ml and mix. This provides the 50 ng/ml SAA standard.
5. Similarly prepare the 25 – 1.56 ng/ml standards by two-fold serial dilution.

[1] Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

HRP CONJUGATE

The HRP conjugate is supplied ready to use.

SAMPLES

In serum, we found SAA levels ranging from 0 to 4500 ng/ml. Optimal dilutions should be determined empirically. However, we found that a 10-fold dilution worked well for most samples. To avoid matrix effects do not use dilutions less than 10-fold. The diluent provided with the kit (SB50-1) must be used for dilution. Do not substitute other buffers.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense of 100 µl of standards and samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker set at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well). If necessary, strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
5. Dispense 100 µl of HRP conjugate into each well.
6. Incubate on a plate shaker set at 150 rpm and 25°C for 45 minutes.
7. Empty and wash the microtiter wells as described in step 4.
8. Dispense 100 µl of TMB into each well.
9. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
10. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix. It is important to make sure that all the blue color changes to yellow.
12. Read absorbance at 450 nm[2] with a plate reader within 5 minutes.

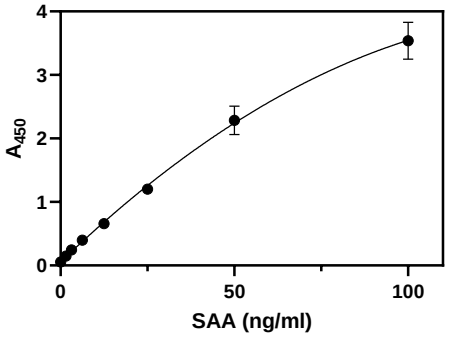
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the SAA concentration. We suggest using a second order polynomial (quadratic) equation.
2. Derive the concentration of SAA in the samples.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
4. If the absorbance values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

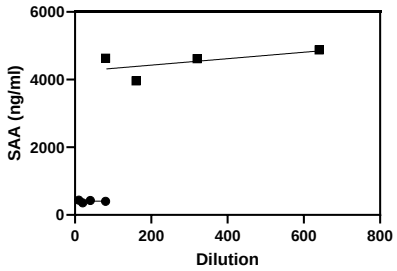
A typical standard curve is shown below. This curve is for illustration only.

SAA (ng/ml)	A_{450}
100	3.538
50	2.286
25	1.201
12.5	0.658
6.25	0.397
3.13	0.244
1.56	0.150
0	0.055



PERFORMANCE

Linearity: To assess the linearity of the assay, two Rainbow Trout serum samples with SAA concentrations of 407 and 4522 ng/ml were serially diluted to give values within range of the assay.



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[2] If absorbance of the high standard is ≥4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.

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