Guinea Pig SAA ELISA SAA-7

INTRODUCTION

Serum amyloid A (SAA), a protein of approximately 12 kDa, is a positive acute phase reactant that is produced in liver and circulates in blood. Serum levels are increased during inflammation and infection.

PRINCIPLE OF THE ASSAY

The assay uses two peptide-specific polyclonal antibodies generated against guinea pig SAA. Standards and samples (100 µl) are incubated in anti-SAA coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µl) is added and incubated for 45 minutes. If SAA molecules are present, they are sandwiched between the capture 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 after 20-minutes by 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 stock
- SAA stock, 1 vial. **Store at -20°C**
- 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 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 -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. 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 (CSD50-1) is formulated for measurement of SAA in serum. It is used for dilution of samples, and for dilution of the HRP conjugate stock. It is supplied ready to use. DO NOT substitute other buffers.

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

STANDARD

1. The stock is lyophilized. It is comprised of recombinant guinea pig SAA in a stabilizing matrix. Reconstitute it with deionized water as described on the vial label and gently mix. Prepare the 6 ng/ml standard as described on the label.
2. Label seven polypropylene tubes as 3, 1.5, 0.75, 0.375, 0.188, 0.094 and 0 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 6 ng/ml SAA standard into the tube labeled 3 ng/ml and mix. This provides the 3 ng/ml SAA standard.
4. Similarly prepare the 1.5 to 0.094 ng/ml standards by two-fold serial dilution.

IMPORTANT – If future use of the stock is intended, the capped vial must be frozen at or below -20°C within 30 minutes of reconstitution.

SAMPLES

The assay is intended for measurement of SAA in guinea pig serum. We found that a dilution of 100-fold worked for healthy serum. Optimal dilutions of guinea pig acute phase serum should be determined empirically.

HRP CONJUGATE

The HRP conjugate is provided as a concentrated stock. Prior to use it must be diluted with Diluent CSD50-1 as described on the vial label. Use 100 µl of the diluted HRP conjugate per well.

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

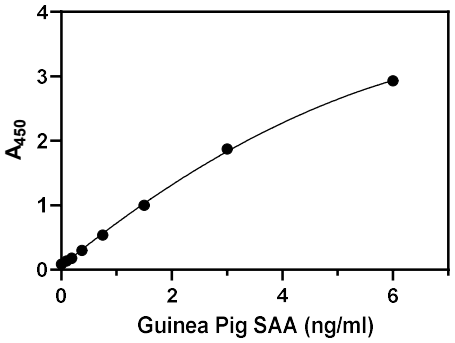
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the SAA concentration. We suggest fitting data second order polynomial 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 original sample.
4. If the absorbance values of samples fall outside the standard curve, samples should be further 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}
6	2.932
3	1.870
1.5	0.999
0.75	0.540
0.375	0.297
0.188	0.180
0.094	0.135
0	0.084

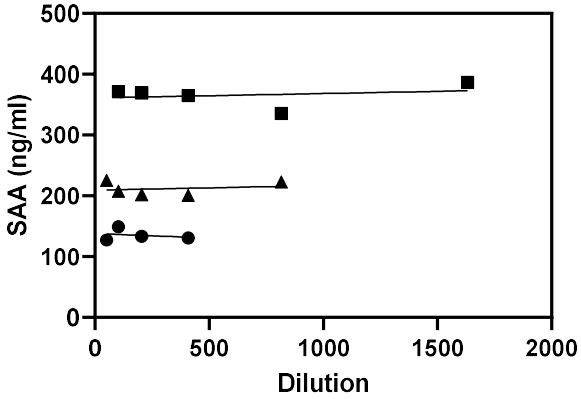


[2] If absorbance of the high standard is ≥4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.

Life Diagnostics, Inc.
124 Turner Lane, West Chester, PA 19380
610-431-7707 - info@lifediagnostics.com.com – www.lifediagnostics.com

PERFORMANCE

Linearity: To assess the linearity of the assay, serum samples with SAA concentrations of 127, 209 and 364 ng/ml were serially diluted with CSD50-1 to give values within range of the assay.



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

Life Diagnostics, Inc.
124 Turner Lane, West Chester, PA 19380
610-431-7707 - info@lifediagnostics.com.com – www.lifediagnostics.com