CHICKEN SERUM AMYLOID A (SAA) ELISA
Life Diagnostics, Inc., Catalog Number: SAA-5

INTRODUCTION

SAA, a protein of approximately 12 kDa, is a positive acute phase reactant that circulates in blood. In chickens SAA gene expression increases >100 fold following infection.[1] Serum levels of SAA have been reported to increase 3-fold during amyloid arthropathy.[2]

PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific chicken SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. Serum samples are first denatured by heating for 1 hour at 60°C. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured samples are diluted. Standards and diluted samples are incubated, in the microtiter wells, together with HRP conjugate 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 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: YD50-1, 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 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 stock vial contains lyophilized heat-treated SAA of known concentration (it must not be incubated at 60°C).

1. Reconstitute the stock with deionized or distilled water as described on the vial label. Mix gently several times over a period of 5 minutes.
2. Label 7 polypropylene tubes as 15, 7.5, 3.75, 1.88, 0.94, 0.47 and 0.23 ng/ml.
3. Into the tube labeled 15 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 15 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 7.5, 3.75, 1.88, 0.94, 0.47 and 0.23 ng/ml.
5. Prepare the 7.5 ng/ml standard by mixing 250 µl of the 15 ng/ml standard with 250 µl of diluent in the tube labeled 7.5 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Unused reconstituted stock should be frozen at or below -20°C if future use is intended.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µl of each serum sample into a polypropylene microcentrifuge tube and tightly seal.
2. Incubate the samples at 60°C in a water bath for one hour.

Dilution

1. After denaturation, dilute the denatured samples at least 10-fold with the diluent provided with the kit.

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.

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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.

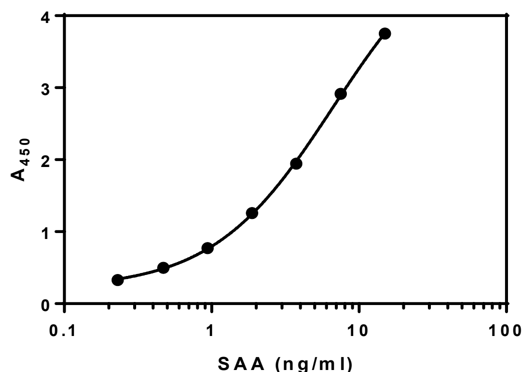
CALCULATION OF RESULTS

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}
15	3.751
7.5	2.916
3.75	1.944
1.88	1.257
0.94	0.770
0.47	0.497
0.23	0.329



ASSAY CHARACTERISTICS

Typical results obtained with six normal chicken serum samples are shown in the following. Samples were heated at 60°C for one hour then diluted as indicated and concentrations (ng/ml) determined. Serum concentrations (SC, µg/ml) were calculated by multiplying the derived concentrations of the samples by their respective dilution factors. Average values, standard deviation (SD) and coefficient of variation (CV) were then calculated.

Sample	Dilution	A_{450}	ng/ml	SC ng/ml	Average (ng/ml)	SD (ng/ml)	CV
LDIC8	10	1.293	1.99	19.86	21.08	2.09	9.9
	20	0.782	0.94	18.83			
	40	0.552	0.56	22.32			
	80	0.371	0.29	23.31			
LDIC9	10	1.463	2.39	23.95	21.48	1.79	8.3
	20	0.859	1.08	21.63			
	40	0.517	0.50	20.16			
	80	0.343	0.25	20.17			
LDIC10	10	2.117	4.28	42.83	44.98	2.24	5.0
	20	1.444	2.35	46.98			
	40	0.906	1.17	46.84			
	80	0.541	0.54	43.27			
LDIC11	10	0.917	1.19	11.92	10.90	0.96	8.8
	20	0.537	0.53	10.69			
	40	0.365	0.28	11.32			
	80	0.243	0.12	9.67			
LDIC12	10	0.773	0.93	9.26	10.55	1.06	10.0
	20	0.572	0.59	11.79			
	40	0.357	0.27	10.88			
	80	0.249	0.13	10.28			
LDIC13	10	2.256	4.76	47.57	44.07	2.55	5.8
	20	1.337	2.09	41.80			
	40	0.850	1.07	42.63			
	80	0.549	0.55	44.27			

REFERENCES

1. Matulova M, et al. Chicken innate immune response to oral infection with Salmonella enterica serovar Entiritidis. Veterinary Research. 44:37 (2013)
2. Alilar AA. et al. Chronic amyloid arthropathy and increased serum amyloid levels in brown layers. Bull Vet Inst Pulawy. 50:557-560 (2006)

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For technical assistance please email us at
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