

DOG SERUM AMYLOID A (SAA) ELISA

Life Diagnostics, Inc., Catalog Number: SAA-4

INTRODUCTION

SAA, a protein of approximately 12 kDa, is a positive acute phase reactant that circulates in blood mostly associated with high density lipoprotein. In dogs, basal serum levels are <2 µg/ml but levels can reach 500 µg/ml during infection, disease and cancer.¹⁻³

PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific dog SAA antibodies; one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. 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

1. Reconstitute the SAA stock as described on the vial label. Mix gently several times over a period of 5 minutes before use.
2. Label 6 polypropylene tubes as 1000, 500, 250, 125, 62.5, and 31.25 ng/ml.
3. Into the tube labeled 1000 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix. This provides the 1000 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 500, 250, 125, 62.5, and 31.25 ng/ml
5. Prepare the 250 ng/ml standard by mixing 250 µl of the 1000 ng/ml SAA standard with 250 µl of diluent in tube labeled 500 ng/ml.
6. Similarly prepare the remaining standards by serial dilution. Unused reconstituted stock should be stored frozen at or below -20°C if future use is intended.

SAMPLE PREPARATION

Serum or plasma samples should be diluted at least 100-fold using the diluent provided with the kit. Optimum dilution factors must be determined empirically.

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.

CALCULATION OF RESULTS

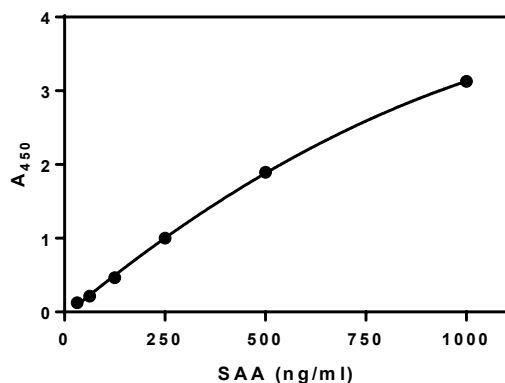
1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.

2. Fit the standard curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum 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}
1000	3.127
500	1.896
250	1.001
125	0.465
62.5	0.215
31.25	0.124



ASSAY CHARACTERISTICS

Typical results obtained with serum from dogs undergoing an acute phase response are shown below. Serum concentrations (SC, $\mu\text{g/ml}$) were calculated by multiplying the derived SAA concentrations of the diluted samples by their respective dilution factors. Average values, standard deviation (SD) and coefficient of variation (CV) were then calculated. SAA concentrations in serum from ten normal dogs were in the range of 0.2 to 2 $\mu\text{g/ml}$ (data not shown).

Sample	Dilution	A450	ng/ml	SC $\mu\text{g/ml}$	Average ($\mu\text{g/ml}$)	SD ($\mu\text{g/ml}$)	CV
D1	500	2.912	888.6	444.3	432.6	19.8	4.6
	1000	1.731	453.1	453.1			
	2000	0.852	211.8	423.6			
	4000	0.400	102.3	409.4			
D2	500	2.432	687.7	343.8	316.0	22.8	7.2
	1000	1.286	325.5	325.5			
	2000	0.594	148.3	296.6			
	4000	0.280	74.5	298.1			

REFERENCES

1. Tecles F, et al. Serum acute phase protein concentrations in dogs with mammary tumors. J Vet Diagn Invest, 21:214-219 (2009)
2. Lowrie M, et al. The role of acute phase proteins in diagnosis and management of steroid-responsive meningitis arteritis in dogs. The Veterinary Journal. 182:125-130 (2009)
3. Christensen MB et al. Canine serum amyloid A (SAA) measured by automated latex agglutination turbidimetry is useful for routine sensitive and specific detection of systemic inflammation in a general clinical setting. J. Vet. Med. Sci. 75(4):459-466 (2013)

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