HORSE SERUM AMYLOID A (SAA) ELISA
Life Diagnostics, Inc., Catalog Number: SAA-14

INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of approximately 12 kDa that is expressed in lever and circulates in blood. Serum levels in horses can increase several hundred-fold because of infection, disease, and tissue injury.[1-3]

PRINCIPLE OF THE ASSAY

The assay uses two different peptide-specific horse 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 one 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: CSD50-1, 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
- Water bath
- 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. 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 before use. The stock does not require heat treatment
2. Label 7 polypropylene tubes as 250, 125, 62.5, 31.25,15.63, 7.81 and 3.91 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the SAA stock vial label. Then add the indicated volume of stock and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml.
5. Pipette 250 µl of the 250 ng/ml SAA standard into the tube labeled 125 ng/ml and mix. This provides the 125 ng/ml SAA standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Unused stock should be stored 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 50-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.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

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.

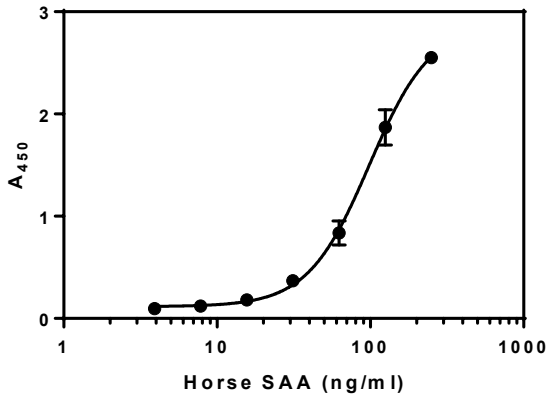
CALCULATION OF RESULTS

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 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}
250	2.544
125	1.661
62.5	0.693
31.25	0.322
15.63	0.171
7.81	0.113
3.91	0.095



ASSAY CHARACTERISTICS

Typical results obtained with four normal horse serum samples are shown in the following table. Samples were heated at 60°C for one hour, then diluted 100 to 800-fold and concentrations (ng/ml) determined. Serum concentrations (SC) were calculated by multiplying the concentrations of the diluted samples by their respective dilution factors. Average values, standard deviation (SD) and coefficient of variation (CV) were then calculated.

Sample	Dilution	A450	ng/ml	SC (ng/ml)	Average (ng/ml)	SD (ng/ml)	CV
1H	100	1.978	232.5	23246	21934	1364	6.2
	200	1.251	114.8	22961			
	400	0.732	52.4	20950			
	800	0.468	25.7	20578			
2H	100	0.950	76.8	7683	8387	616	7.3
	200	0.654	44.1	8828			
	400	0.424	21.6	8650			
	800						
3H	100	2.174	271.7	27172	28915	1912	6.6
	200	1.513	152.6	30512			
	400	0.877	68.4	27349			
	800	0.596	38.3	30628			
4H	100	1.596	165.6	16561	17606	1395	7.9
	200	1.115	97.0	19405			
	400	0.624	41.1	16447			
	800	0.434	22.5	18011			

REFERENCES

1. Satue K, Calvo A and Gardon JC. Factors influencing serum amyloid type A (SAA) concentrations in horses. Open Journal of Veterinary Medicine. 3:58-66 (2013)
2. Cywinska A. et al. Serum amyloid A (SAA) concentration after training sessions in arabian race and endurance horses. BMC Veterinary Research. 9:91 (2013)
3. Pepys MB, Baltz ML and Tennent GA. Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. Equine Vet. J. 21(2):106-109 (1989)

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