

INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of approximately 12 kDa. Serum levels increase due to inflammation and infection in many species. In elephants, serum levels of 0 - 47.5 µg/ml have been reported for healthy elephants (ref 1). Levels up to 410 µg/ml were found in sick elephants.

The assay uses two elephant SAA peptide-specific antibodies. One is used for solid phase immobilization (microtiter wells). The other, conjugated to HRP, is used for detection. Diluted serum samples and standards are incubated in microtiter wells together with HRP conjugate for one hour. If present, SAA molecules are 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

Materials provided with the kit:

- Anti-SAA coated plate (12 x 8-well strips)
- HRP Conjugate: 11 ml
- SAA stock
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 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
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

The kit should be stored at 4°C. 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

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 is provided ready to use. Do not substitute other buffers.

STANDARD

1. The SAA stock consists of an elephant SAA polypeptide lyophilized in a stabilizing matrix. Prepare the 250 ng/ml standard as described on the vial label.
2. Label seven polypropylene tubes as 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 250 ng/ml SAA standard into the tube labeled 125 ng/ml and mix. This provides the 125 ng/ml SAA standard.
4. Similarly prepare the remaining standards by two-fold serial dilution.

Use the standards within 30 minutes. The reconstituted 250 ng/ml stock should be stored at -20°C for optimum stability.

SAMPLES

This kit was designed for measurement of SAA in serum. We found levels ranging from 5 µg/ml in healthy animals to >500 µg/ml in serum from sick elephants. We suggest testing each sample at dilutions of 250-fold and 10,000-fold to obtain at least one value within range of the standard curve.

1. A 250-fold dilution can be obtained by mixing 2.0 µl of serum with 498 µl of diluent.
2. A 10,000-fold dilution can be obtained by mixing 12.5 µl of the 250-fold diluted sample with 487.5 µl of diluent.

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 samples into the wells. We recommend that standards and samples be run in duplicate.
3. Add 100 µl of HRP-conjugate to 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 residual droplets if present.
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.

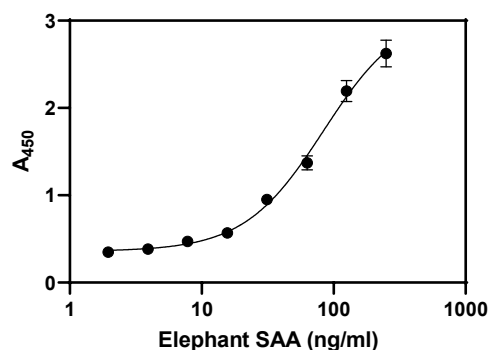
RESULTS

1. Using graphing software, construct a standard curve by plotting absorbance values of the standards versus log₁₀ of the SAA concentration.
2. Fit the standard curve to a four-parameter logistic equation and derive concentrations for the samples.
3. Multiply the derived concentration of samples by the dilution factor to determine the concentration in the original sample.
4. If the A₄₅₀ values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested. Ideally, A₄₅₀ values of samples should fall within the mid-range of the standard curve.

TYPICAL STANDARD CURVE

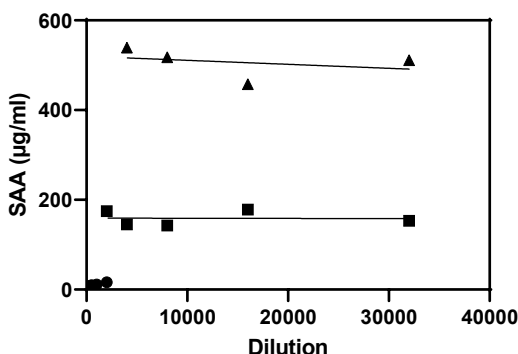
A typical standard curve with absorbance at 450 nm on the Y-axis against SAA concentrations on the X-axis is shown below. This curve is for illustration only.

SAA (ng/ml)	A ₄₅₀
250	2.625
125	2.195
62.5	1.371
31.25	0.951
15.63	0.568
7.81	0.471
3.91	0.383
1.95	0.349



PERFORMANCE

Linearity: To assess the linearity of the assay, three samples containing SAA at concentrations of 11, 160 and 507 µg/ml were serially diluted with diluent to give values within range of the standard curve.



SAMPLES

1. Isaza R., et al. Reference intervals for acute phase protein and serum protein electrophoresis values in captive Asian elephants (*Elephas maximus*). *Journal of Veterinary Diagnostic Investigation*. 26(5): 616-621 (2014)

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For technical assistance please email us at info@vetbiomarkers.com