MONKEY SERUM AMYLOID A (SAA) ELISA TEST KIT
Life Diagnostics, Inc., Catalog Number: 3400-2

Enzyme Immunoassay for the Quantitative Determination of Monkey Serum Amyloid A (SAA) in Serum

FOR RESEARCH USE ONLY

INTRODUCTION

SAA is an acute phase serum protein that can be elevated approximately 70-fold in monkeys (ref. 1). As is the case in humans, measurement of SAA provides an excellent biomarker of inflammation and disease.

PRINCIPLE OF THE TEST

The monkey SAA ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses two different affinity purified peptide-specific polyclonal anti-monkey SAA antibodies, one for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), for detection. The test sample is first denatured by heating serum for 1 hour at 60°C. The denaturing step dissociates SAA from interfering factors. Subsequently, the denatured sample is diluted and incubated in the microtiter wells together with the 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-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature during which a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of SAA is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-monkey SAA coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 ml
- SAA standard stock (0.20 ml, lyophilized)[1]
- Wash Buffer (20x stock, 50 ml)
- Sample Diluent (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer
- 60°C water bath
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm.
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

Upon receiving the kit please store the SAA standard in a freezer at or below -20°C. The remaining components of the kit should be stored in a refrigerator at 2-8°C. It is important that the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18- 25°C) before use.
2. Please take the time to completely read and understand this kit insert before starting your assay. Donquhesitate to contact Life Diagnostics by telephone or email should you require technical assistance or clarification.

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.

SAMPLE PREPARATION

Denaturation

1. Dispense 100 µl of serum into a polypropylene microcentrifuge tube.
2. Repeat this procedure for each sample to be tested.
3. Incubate the samples at 60°C in a water bath for one hour.

Dilution

1. After denaturation, dilute 1.0 µl of denatured sample with 249 µl of sample diluent.
2. Repeat this procedure for each sample to be tested.

This procedure gives a 250-fold dilution of the original sample and presents SAA in a form that is recognizable by the antibodies used in the kit.

STANDARD PREPARATION

The standard vial contains lyophilized heat-treated SAA of known concentration (the standard <u>must not</u> be incubated at 60°C).

1. Reconstitute the SAA standard stock by addition of 200 µl of de-ionized or distilled water. Mix gently several times over a period of 5 minutes.
2. Label 6 polypropylene tubes as 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml.
3. Into the tube labeled 25 ng/ml, pipette the volume of standard diluent detailed on the SAA standard vial label. Then add the indicated volume of SAA standard (also shown on the vial label) and mix gently. This provides the working 25 ng/ml standard.

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[1] The SAA standard used in this kit is of non-monkey origin. It behaves identically to old-world monkey SAA. The use of a non-monkey standard allows export of the kit without the requirement for CITES documentation.

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4. Dispense 250 µl of standard diluent into the tubes labeled 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml.
5. Pipette 250 µl of the 25 ng/ml SAA standard into the tube labeled 12.5 ng/ml and mix. This provides the working 12.5 ng/ml SAA standard.
6. Similarly prepare the 6.25, 3.125, 1.56 and 0.78 ng/ml standards by serial dilution.

Please Note: Unused reconstituted reference standard stock should be stored frozen at or below -20°C if future use is intended (it is stable for at least one week at 4°C).

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and denatured/diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
3. Add 100 µl of HRP conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for one hour.
5. Wash and empty the microtiter wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
7. Dispense 100 µl of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for 20 minutes.
9. 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 the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

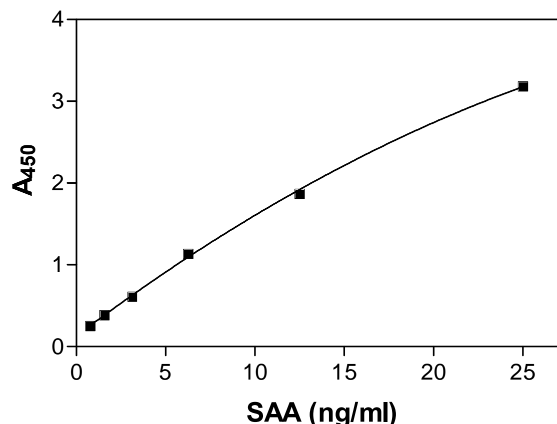
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of SAA in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of SAA in the serum sample.
5. If available, PC graphing software should be used for the above steps. We find that good a good fit of the data are obtained to either a two site binding equation or a second order polynomial equation.
6. If the A_{450} values of samples fall outside the standard curve when tested at a dilution of 250 fold, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A representative standard curve with optical density readings at 450nm on the Y axis against SAA concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

SAA (ng/ml)	Absorbance (450 nm)
25	3.182
12.5	1.870
6.25	1.139
3.125	0.609
1.56	0.384
0.78	0.252

Representative Monkey SAA Standard Curve



REFERENCES

1. Hsieh FY, et.al. Toxicological protein biomarker analysis . an investigative one-week single dose intravenous infusion toxicity and toxicokinetic study in cynomolgus monkeys using an antibody-cytotoxic conjugate against ovarian cancer. Pharmaceutical Research. 25: 1309-1317 (2008)

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