KAMIYA BIOMEDICAL COMPANY

Monkey Serum Amyloid A ELISA

For the quantitative determination of serum amyloid A (SAA) in monkey serum

Cat. No. KT-496

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY***® Monkey Serum Amyloid A ELISA is an enzyme immunoassay for the quantitative determination of serum amyloid A (SAA) in monkey serum. For research use only.

INTRODUCTION
SAA is an acute phase serum protein that can be elevated up to 400-fold in monkeys. As is the case in humans, measurement of SAA provides an excellent biomarker of inflammation and disease.

PRINCIPLE
The assay uses two different peptide-specific monkey 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. Calibrators 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, and absorbance is measured at 450 nm. The concentration of SAA is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- SAA antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- SAA Calibrator (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent, 50 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software
- Plate washer

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 de-ionized water.

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 1.0 µL of denatured sample with 249 µL of diluent.

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.

CALIBRATOR PREPARATION

The calibrator vial contains lyophilized heat-treated SAA of known concentration (it must not be incubated at 60°C).

1. Reconstitute the calibrator with de-ionized or distilled water as described on the vial label. 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 484.66 µL of diluent. Then add 15.34 µL of calibrator and mix. This provides the 25 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 12.5, 6.25, 3.125, 1.56 and 0.78 ng/mL.
5. Prepare the 12.5 ng/mL calibrator by mixing 250 µL of the 25 ng/mL SAA calibrator with 250 µL of diluent in the tube labeled 12.5 ng/mL.
6. Similarly prepare the remaining calibrators by serial dilution.

Unused reconstituted calibrator 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 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 calibrators and samples into the wells (we recommend that calibrators 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.

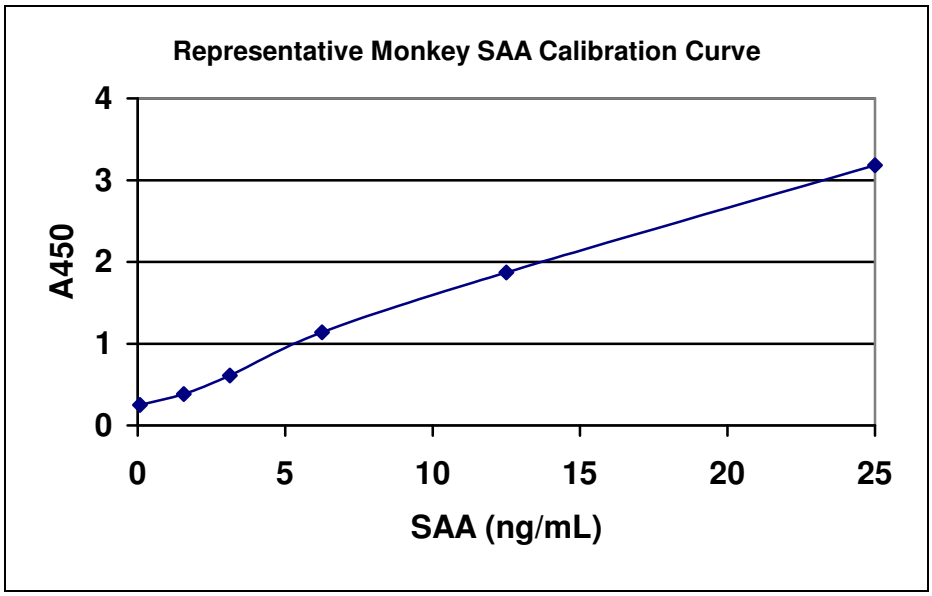
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.
2. Fit the calibration 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 calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration 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}
25	3.182
12.5	1.870
6.25	1.139
3.125	0.609
1.56	0.384
0.78	0.252



STORAGE

The SAA calibrator should be stored at or below -20℃ for optimum stability. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

FOR RESEARCH USE ONLY

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