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 approximately 70-fold in monkeys. As is the case in humans, measurement of SAA provides an excellent biomarker of inflammation and disease.

PRINCIPLE
The **K-ASSAY®** Monkey Serum Amyloid A 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.

COMPONENTS
- Anti-monkey SAA antibody coated microtiter plate with 96 wells (provided as 12 x 8-well strips)
- HRP Conjugate Reagent, 11 mL
- Monkey SAA Calibrator (0.20 mL, lyophilized)
  Note: The SAA Calibrator used in this kit is of non-monkey origin. It behaves identically to old-world monkey SAA. The use of a non-monkey calibrator allows export of the kit without the requirement for CITES documentation.
- Wash Buffer (20X), 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 de-ionized water
- Polypropylene tubes
- Vortex mixer
- 60°C water bath
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate reader capable of measuring absorbance at 450 nm
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25°C) before use.
Please take the time to completely read and understand this kit insert before starting your assay. Don't hesitate to contact us by telephone or e-mail 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 de-ionized 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.

CALIBRATOR PREPARATION

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

1. Reconstitute the Monkey SAA Calibrator by addition of 200 µL of de-ionized or distilled water. Mix gently several times over a period of 5 minutes. (**The reconstituted calibrator remains stable for at least 7 days at 4°C but should be aliquoted and frozen at or below -20°C after reconstitution if use beyond this time is intended).**
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 472.4 µL of calibrator diluent. Then add 27.62 µL of SAA calibrator and mix gently. This provides the working 25 ng/mL calibrator.
4. Dispense 250 µL of calibrator 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 calibrator into the tube labeled 12.5 ng/mL and mix. This provides the working 12.5 ng/mL calibrator.
6. Similarly prepare the 6.25, 3.125, 1.56 and 0.78 ng/mL calibrators by serial dilution.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and denatured/diluted samples into the wells (we recommend that calibrators 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 adsorbent 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

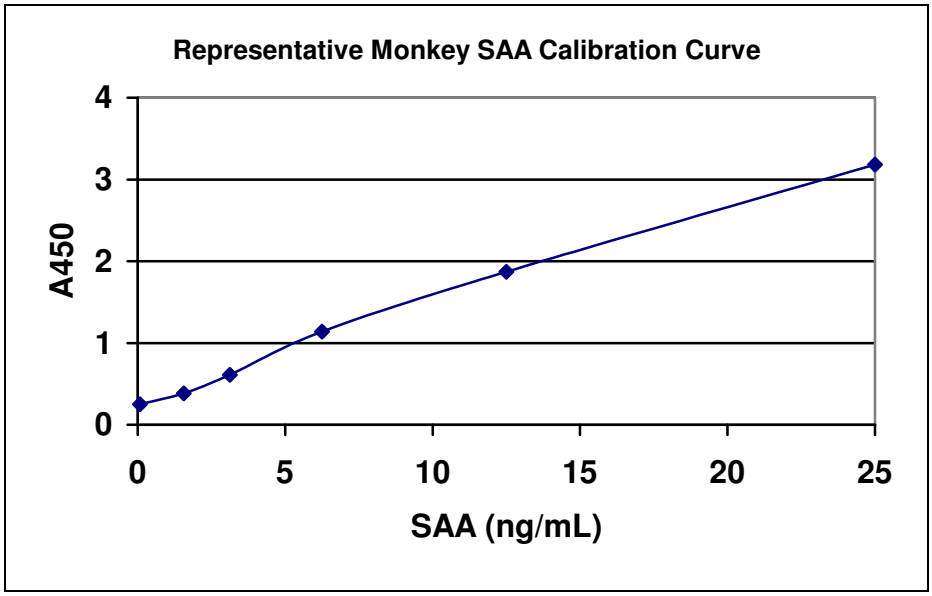
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration 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 calibration curve.
4. Multiply the derived concentration 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 a good fit of the data is obtained with either a two site binding equation or a second order polynomial equation.

6. If the A_{450} values of samples fall outside the calibration curve when tested at a dilution of 250 fold, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against SAA concentration 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 calibration 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



STORAGE

Upon receiving the kit please store the SAA calibrator in a freezer at or below -20℃. The remaining components of the kit should be stored in a refrigerator at 4℃. It is important that the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit will remain stable until the expiration date provided that the components are stored as described above.

FOR RESEARCH USE ONLY

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