Catalog #80659

V.1

Mouse Serum Amyloid A (SAA) ELISA Kit Instructions

For the quantitative determination of SAA in mouse serum and plasma

Catalog #80659
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
1536 Brook Drive, Suite A
Downers Grove, IL 60515, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order Online: www.crystalchem.com

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A. Intended Use

The Mouse SAA ELISA kit is for the quantitative determination of SAA in mouse serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures. For in Vitro Use Only.

B. Introduction

Serum Amyloid A, SAA, is part of a lipid binding protein family produced primarily by the liver and plays several roles in the body including transport of cholesterol and recruitment of immune cells. The proteins are found in most vertebrates and invertebrates suggesting some vital function. In mice, three main forms are known: SAA1, SAA2, and SAA3. SAA is currently being studied as a marker for inflammation and other disease activity.

C. Principle of the Assay

The Mouse SAA ELISA kit is a double antibody sandwich ELISA. In the assay, the SAA present in the sample binds with anti-SAA antibodies adsorbed to the surface of the microplate. In the following step, another specific anti-SAA antibody binds, in turn, to the immobilized SAA. The second antibody is conjugated with horseradish peroxidase (HRP). In the closing substrate reaction, the SAA levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Mouse SAA ELISA kit, **remove the calibrator and store it frozen. Store the remaining components at 2-8°C** and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 CONTENTS OF KIT

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
CAL	Mouse SAA Calibrator	1 x 1 vial
DIL	Diluent (5X Concentrate)	1 x 50 mL
ENZ CONJ	Enzyme Conjugate (100X Concentrate)	1 vial/150 µL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Standard laboratory glassware for buffer and reagent preparation
Distilled or deionized water
Polypropylene microtubes
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Each calibrator and sample should be assayed in duplicate.
2. Given the sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
3. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples with excessive hemolysis should not be used. *Note: Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.*

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-Coated microplate
   Provided as ready to use. Protect from moisture.
2. Calibrator
   The calibrator needs to be reconstituted with 1 mL of distilled or dionized water (per vial), immediately before use to yield a 15 µg/mL solution. Prepare working standards as described in **I.2**, by diluting with 1x Diluent. On initial use, store the remaining calibrator at -20°C in aliquots as required.

3. Diluent (5X Concentrated)
   The Diluent has to be diluted 1:5 with distilled or deionized water prior to use. For example, 50 mL of 5X Diluent must be diluted with 200 mL of distilled or deionized water. Diluent is stable for at least one week after dilution stored at 2-8°C. Prepare only as needed.
4. Enzyme Conjugate (100X Concentrated)
   The Enzyme Conjugate has to be diluted 1:100 with 1X Diluent prior to use. For example, 10 µL of the enzyme must be diluted with 990 µL of 1X Diluent. The Enzyme Conjugate is stable for up to 1 hour when stored in the dark. Accordingly, Enzyme Conjugate should be prepared only as needed just prior to use.
5. Wash Buffer (20X Concentrated)
   The Wash Buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of 20X Wash Buffer must be diluted with 950 mL of distilled or deionized water. Wash Buffer is stable for at least one week after dilution at room temperature or at 2-8°C.
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Preparation of working standards

1. Pipette 120 µL of the calibrator into 780 µL of 1x Diluent into a polypropylene microtube labeled 2000 ng/mL, and mix thoroughly.
2. Pipette 250 µL of 1X Diluent into six polypropylene microtubes labeled 1000, 500, 250, 125, 62.5, and 31.25 ng/mL.
3. Dispense 250 µL of the 2000 ng/mL standard into the 1000 ng/mL microtube, and mix thoroughly.
4. Dispense 250 µL of the 1000 ng/mL standard into the 500 ng/mL microtube, and mix thoroughly.
5. Dispense 250 µL of the 500 ng/mL standard into the 250 ng/mL microtube, and mix thoroughly.
6. Dispense 250 µL of the 250 ng/mL standard into the 125 ng/mL microtube, and mix thoroughly.
7. Dispense 250 µL of the 125 ng/mL standard into the 62.5 ng/mL microtube, and mix thoroughly.
8. Dispense 250 µL of the 62.5 ng/mL standard into the 31.25 ng/mL microtube, and mix thoroughly.
9. Dispense 500 µL of 1X Diluent into one polypropylene microtube labeled 0 ng/mL. You now have working standards of 2000,1000, 500, 250, 125, 62.5, 31.25, and 0 ng/mL.

Please note: Working standards should be prepared immediately prior to use.

I.3. Dilution of samples

Samples need to be diluted with 1X Diluent for use with the assay.

Serum/Plasma Samples: A sample dilution of 1:1000 using 5 µL of sample is generally suitable. To prepare the 1:1000 dilution, mix 5 µL of sample with 495 µL of 1X Diluent to yield a 1:100 dilution. Next, remove 30 µL of the dilution, and add to 270 µL of 1x Diluent to prepare the 1:1000 dilution.

Since SAA levels can vary, dilution ratios may need to be adjusted as appropriate.

I.4. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or working standard.
2. Incubate plate for 60 mins at room temperature. Keep plate covered and level.
3. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. In each well, add 100 µL of diluted Enzyme Conjugate and mix well by repeated pipetting.
5. Incubate plate for 30 mins at room temperature. Keep plate covered in the dark and level.
6. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Substrate Solution in each well.
8. Incubate plate for 10 mins in dark room at room temperature.
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance as soon as possible, and within 30 mins, using a plate reader (measure A_{450} values and subtract A_{630} values).

I.5. Determining the SAA concentration

1. Using computer software, construct the SAA calibration curve by plotting the mean absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding SAA concentration on the X axis. A four parametric logistics (4-PL) curve fit is suitable for the evaluation. ***Note:*** *A calibration curve should be plotted every time the assay is performed.*
2. Mouse SAA concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (i.e. 1000) to obtain the final SAA concentration (expressed in ng/mL).
   Note: *Samples with high mouse SAA concentrations (ie. fall above the range of the assay) should be further diluted and rerun.*

J. Performance characteristics

J.1. Assay range

The Mouse SAA ELISA Kit has an assay range from 31.25 - 2,000 ng/mL.

J.2. Sensitivity

The sensitivity of this assay is 12 ng/mL.

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