Mouse Serum Amyloid P ELISA

For quantitative determination Serum Amyloid P (SAP) in mouse serum and plasma.

For Research Use Only. Not For Use In Diagnostic Procedures.

Please See Appendix A for Reference Serum Information.

Catalog Number: 41-SAPMS-E01
Size: 96 Wells
Version: 3 L78 – ALPCO 2.0

INTENDED USE

The Mouse Serum Amyloid P (SAP) ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring SAP in serum and plasma samples of mice.

INTRODUCTION

Mouse serum amyloid P (SAP) is a major acute phase protein that circulates as an ~230 kDa glycoprotein. It is composed of 10 identical subunits arranged as two cyclic pentameric discs and contains ~10% carbohydrate. Research demonstrates that although the basal levels of SAP may vary significantly in different inbred mouse strains, SAP is a major acute phase protein in all strains. During acute chemical, physical or inflammatory stimulus, its concentration can increase 50-100 fold within 24-48 hours. Changes can readily be detected in 4-6 hours. Measurement of the concentration of SAP is indicative of the extent and severity of an inflammatory stimulus and can be used to research various modalities of treatment.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the SAP present in samples reacts with the anti-SAP antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-SAP antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound SAP. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of SAP in the sample tested, thus, the absorbance, at 450 nm, is a measure of the concentration of SAP in the test sample. The quantity of SAP in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

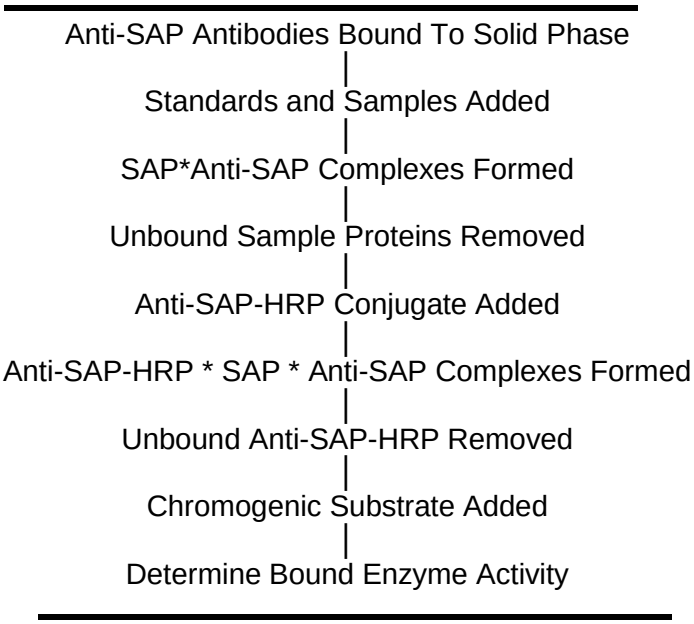

Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE (Assay Buffer)
   One bottle containing 50 mL of a 5X concentrated diluent assay buffer.

2. WASH SOLUTION CONCENTRATE
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. ENZYME-ANTIBODY CONJUGATE 100X
   One vial containing 150 µL of affinity purified anti-Mouse serum amyloid P antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN-SUBSTRATE SOLUTION
   One vial containing 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION
   One vial containing 12 mL 0.3 M sulfuric acid. **WARNING: Avoid contact with skin.**

6. ANTI-MOUSE SERUM AMYLOID P ELISA MICROPLATE
   Twelve removable eight (8) well microwell strips in well holder frame. Each well is coated with affinity purified anti-Mouse serum amyloid P.

7. MOUSE SERUM AMYLOID P CALIBRATOR
   One vial containing a lyophilized mouse serum amyloid P calibrator.

FOR RESEARCH USE ONLY

REAGENT PREPARATION

1. DILUENT CONCENTRATE
   The Diluent Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O).

2. WASH SOLUTION CONCENTRATE
   The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. ENZYME-ANTIBODY CONJUGATE
   Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN-SUBSTRATE SOLUTION
   Ready to use as supplied.

5. STOP SOLUTION
   Ready to use as supplied.

6. ANTI-MOUSE SERUM AMYLOID P ELISA MICROPLATE
   Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

7. MOUSE SERUM AMYLOID P CALIBRATOR
   Please refer to the certificate of analysis for the preparation of the mouse serum amyloid P calibrator.

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. ENZYME-ANTIBODY CONJUGATE
   Undiluted horseradish peroxidase anti-serum amyloid P conjugate should be stored at 4-8°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. CHROMOGEN-SUBSTRATE SOLUTION
   The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION
   The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. ANTI-MOUSE SERUM AMYLOID P ELISA MICROPLATE
   Anti-Mouse serum amyloid P coated wells are stable until the expiration date and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

7. MOUSE SERUM AMYLOID P CALIBRATOR
   The lyophilized mouse serum amyloid P calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and **are stable for up to 1 hour.**

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

SAMPLE COLLECTION AND HANDLING

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. Care should be taken to minimize hemolysis, as excessive hemolysis can impact the results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions
   For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives
   No additives or preservatives are necessary to maintain the integrity of the sample. Avoid azide contamination.

3. Known interfering substances
   Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

MATERIAL PROVIDED See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 1000µL) for making and dispensing dilutions
- Test tubes
- Microtiter washer/aspirator
- Distilled or Deionized H_2O
- Microtiter Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge (for sample Collection)
- Anticoagulant (for plasma sample collection)

ASSAY PROTOCOL

DILUTION OF SAMPLES

The assay for quantification of mouse serum amyloid P in samples requires that each test sample be diluted before use. For a single step determination, a dilution of 1:1500 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. **If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.**

1. To prepare a 1:1500 dilution of sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives a 1:100 dilution. Next, dilute the 1:100 samples by transferring 20 µL, to 280 µL of 1X diluent. This yields a 1:1500 dilution of the sample. Mix thoroughly at each stage.

PROCEDURE

1. **Bring all reagents to room temperature before use.**

2. Pipette 100 µL of:
   Standard 0 (0.0 ng/mL) in duplicate
   Standard 1 (6.25 ng/mL) in duplicate
   Standard 2 (12.5 ng/mL) in duplicate
   Standard 3 (25 ng/mL) in duplicate
   Standard 4 (50 ng/mL) in duplicate
   Standard 5 (100 ng/mL) in duplicate
   Standard 6 (200 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre-designated wells.

4. Incubate the microtiter plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for sixty (60 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 µL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the standards, construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the mouse serum amyloid P concentration in the original samples.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, accuracy of reagent and sample pipetting, washing technique, incubation time or temperature.

3. Do not mix or substitute reagents with those from other lots or sources.

Appendix A – Reference Serum Information

One vial containing a Reference Serum is included with this kit. Please refer to the enclosed Product Profile Sheet for lot-specific information. Please note the following:

1. The Reference Serum is stable until the expiry date.

2. The Reference Serum should be diluted as appropriate to fit within the standard range curve. Refer to the “Dilution of Samples” section of the protocol for instructions.

3. While pipetting the samples (Procedure section), also pipette the Reference Serum in duplicate.