MOUSE SERUM AMYLOID P (SAP) ELISA TEST KIT
Life Diagnostics, Inc., Catalog Number: SAP-1

INTRODUCTION

SAP is a member of the pentraxin family of acute phase proteins that includes C-reactive protein. It circulates in blood as a decamer of a single polypeptide chain of mwt 25 kDa. It is a positive acute phase protein in all strains of mice. Levels may increase 50 to 100-fold during the acute phase response but basal levels vary with strain (refs. 1-3). SAP is an excellent acute phase biomarker in mice.

PRINCIPLE OF THE ASSAY

The mouse SAP ELISA uses two peptide-specific antibodies developed at Life Diagnostics, Inc. that recognize different epitopes on mouse SAP. One is used for solid phase immobilization and the other, conjugated to horseradish peroxidase (HRP), is used for detection. Diluted serum samples (100 µl) are incubated in the antibody-coated microtiter wells together with HRP conjugate (100 µl) for one hour. As a result, SAP molecules are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured at 450 nm. The concentration of SAP is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-mouse SAP coated 96-well microtiter (12x8 wells)
- Anti-mouse SAP HRP Conjugate, 11 ml
- SAP stock, 1 vial (lyophilized)[1]
- Diluent; YD50-1, 50 ml
- 20x Wash Solution; TBS50-20, 50 ml
- TMB; TMB11-1, 11 ml
- Stop Solution; SS11-1, 11 ml

Materials required but not provided:

Precision pipettes and tips

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or microcentrifuge tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker (25°C/150 rpm)
- Plate washer
- Plate reader at capable of measuring at 450 nm
- Graphing software

STORAGE

Upon receiving the kit, please store the SAP 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 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 (25°C) before use.
2. Please take the time to completely read and understand this kit insert before starting your assay. Don't hesitate 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

Samples should be diluted at least 40-fold in diluent. Optimum dilutions should be determined empirically.

STANDARD PREPARATION

1. Reconstitute the SAP stock as described on the vial label. Mix gently several times before use.
2. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the SAP stock vial label. Then add the indicated volume of SAP stock and mix gently. This provides the working 250 ng/ml standard.
4. Dispense 250µl of diluent into the tubes labeled 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
5. Pipette 250 µl of the 250 ng/ml SAP standard into the tube labeled 125 ng/ml and mix. This provides the 125 ng/ml SAP standard.
6. Similarly prepare the remaining 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.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and 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 (25°C)[2] 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 into each well.

[1] The SAP standard was calibrated using recombinant mouse SAP from an independent laboratory.

[2] The ELISA was validated using a shaking incubator at 25°C and 150 rpm. Lower temperatures and/or mixing speeds will give lower absorbance values.

8. Gently mix on an orbital micro-plate shaker at 150 rpm at room temperature (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.

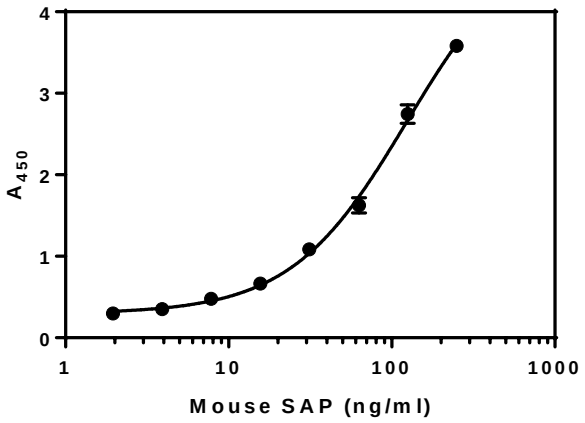
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A representative standard curve with optical density readings at 450 nm on the Y-axis against SAP 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.

SAP (ng/ml)	A_{450}
250	3.582
125	2.745
62.5	1.625
31.25	1.086
15.63	0.664
7.81	0.479
3.91	0.352
1.95	0.298



REFERENCES

1. Baltz ML. et al. Differences in the acute phase responses of serum amyloid P-component and C3 to injections of casein or bovine serum albumin in amyloid-susceptible and –resistant mouse strains. Clin. Exp. Immunol. 39:355-360 (1980)
2. Mortensen RF. Beisel K, Zeleznik NJ and Phong TL. Acute phase reactants of mice. II. Strain dependence of serum amyloid P-component (SAP) levels and response to inflammation. J. Immunol. 130(2): 885-889 (1983)
3. Baltz ML, Dyck RF and Pepys M. Studies of the in vivo synthesis and catabolism of serum amyloid P component (SAP) in the mouse. Clin. Exp. Immunol. 59:235-242 (1985)

Rev 121718

For technical assistance please email us at
techsupport@lifediagnostics.com