人と科学のステキな未来へ
コスモ・バイオ株式会社

MOUSE SERUM AMYLOID P ASSAY

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat. No. 151SAP01M-96

SERUM AMYLOID P

Serum amyloid P (SAP) component is a major acute-phase reactant in mice produced by the liver in conditions of inflammation, bacterial infection, and tissue damage and is responsible for establishing long-term humoral immunity. The expression of mouse SAP can vary widely in different mouse strains, but increases in all strains in response to external stimuli, such as interleukin-6 and lipopolysaccharide[1]. Mouse SAP (mSAP) is comprised of 10 identical subunits that form two pentameric discs that are linked non-covalently. mSAP binds to phosphorylethanolamine[2] in a calcium dependent manner and forms the basis for this assay. Quantification of mouse SAP is particularly useful in assessing disease activity.

INTENDED USE

The HELICA Mouse SAP ELISA is a sandwich-based enzyme-linked immunoassay intended for the detection and quantification of mouse SAP in mouse serum or plasma.

ASSAY PRINCIPLE

The HELICA Mouse SAP ELISA is species specific and provides a quantitative assessment of SAP levels in mouse serum or plasma. The HELICA Mouse SAP assay is a sandwich-based enzyme-linked immunoassay in which the microtiter plates are coated with phosphorylethanolamine. After samples are prepared following the listed specimen collection and preparation procedure, they are applied to the antigen coated plate alongside the prepared standards. After incubation, the wells are decanted and washed to remove unreacted serum or plasma proteins. Rabbit anti-mouse SAP antibody is added and reacts with the SAP bound to the plate. Following another incubation period, the wells are decanted and washed to remove unreacted antibody. HRP conjugated goat anti-rabbit Fc antibody is added and reacts with the rabbit anti-mSAP antibody. Following another incubation period, the wells are decanted and washed to remove unreacted antibody. A hydrogen peroxide substrate with TMB as a chromogen is added to start color development. The intensity of the color is directly proportional to the amount of mouse SAP in the sample. Therefore, the greater the intensity of the blue color, the higher the mouse SAP concentration in the sample. The reaction is interrupted with a stop solution that turns the blue product yellow. The absorbance is read at a wavelength of 450nm on a spectrophotometer or plate reader.

REAGENTS PROVIDED

1 X Pouch	**Antigen Coated Microwell Plate**		96 wells (12 eight well strips) in a microwell holder coated with phosphorylethanolamine, *Ready-to-Use.*
1 X Vial	**Mouse SAP Standard (100X)**	Green Cap	0.13mL of mouse serum with elevated SAP concentration at 20µg/mL. Dilute standard stock 100X in washing buffer followed by four 2.5-fold serial dilutions to prepare standards at the following concentrations: 200.0, 80.0, 32.0, 12.8, and 5.12ng/mL.
1 X Vial	**Anti-Mouse SAP Antibody (100X)**	Pink Cap	0.13mL of 100X rabbit anti-mouse SAP-IgG protein in buffer with preservative. Dilute conjugate in washing buffer to 1X prior to use.
1 X Vial	**Anti-Rabbit Conjugate (100X)**	Amber Cap	0.13mL of 100X goat anti-rabbit IgG conjugated to HRP in buffer with preservative. Dilute conjugate in washing buffer to 1X prior to use.
1 X Bottle	**Substrate Reagent**	Blue Cap	12mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use.*
1 X Bottle	**Stop Solution**	Red Cap	12mL Acidic Solution, *Ready-to-Use.*
1 X Pouch	**Washing Buffer**		Tris with 0.05% Tween20, bring to 1L with distilled water and store refrigerated.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Wash bottle
- Dilution tubes
- Pipettor with tips: 2µL to 1000µL
- Adhesive cover for microplate
- Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19º-27ºC) before use.
2. Store reagents at 2º-8ºC, and never freeze kit components.
3. Do not use the kit beyond the expiration date. Kits are stable for 6 months.
4. Do not return unused reagents back into their original vials or bottles.
5. Do not interchange kit components between different lots of the same assay.
6. Adhere to all time and temperature conditions stated in the procedure.
7. Never pipette reagents or samples by mouth.
8. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
9. The standard serum and conjugate have not been screened for infectious agents, though an anti-microbial preservative, Proclin-300, has been added to limit microbial growth. Since no testing can assure the absence of infectious agents, consider all materials, containers, and devices that are exposed to sample, standard, and conjugate to be contaminated with mouse serum proteins. Wear protective gloves and safety glasses when using this kit.
10. Dispose of all materials, containers, and devices in the appropriate receptacle after use.
11. HRP-labeled conjugate and TMB substrates are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

SPECIMEN COLLECTION AND PREPARATION

Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. Alternatively, plasma extracted from blood drawn in heparin, EDTA, or ACD-containing tubes is acceptable. If testing is to be delayed longer than 1 day, freezing the sample at -20°C or below is recommended.

Upon specimen collection, dilute the sample 1:200 in Tris-Tween wash buffer (i.e. add 5µL of sample to 995µL of wash buffer). Follow this with an additional dilution of 1:100 in Tris-Tween (i.e. add 5µL of the 1:200 diluted sample to 495 µL of wash buffer). The final dilution for use in calculation is 1:20,000.

ASSAY PROCEDURE

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay if more than 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. Reconstitute the Tris-Tween packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Q.S. to 1 Liter with distilled or deionized water and store refrigerated when not in use.
2. Dilute 100X mSAP standard stock to 1X working concentration in Tris-Tween to prepare 200.0ng/mL standard. Prepare remaining standards by serially diluting standards 2.5-fold four times to yield 80.0, 32.0, 12.8, and 5.12ng/mL. Use Tris-Tween wash buffer for 0.0ng/mL standard. Consider the following dilution scheme as a guide. The volumes detailed below contain enough standard to run in duplicate wells.

Standard Concentration (ng/mL)	Tris-Tween Volume	Volume Transferred	Total Volume	Final Volume *After serial dilution
200.0	495µL	5µL	500µL	300µL
80.0	300µL	200µL	500µL	300µL
32.0	300µL	200µL	500µL	300µL
12.8	300µL	200µL	500µL	300µL
5.12	300µL	200µL	500µL	500µL

3. Using a new pipette tip for each, add 100µL of each standard and prepared sample into the appropriate wells. Samples and standards can be run in duplicate if desired. Incubate at room temperature for 1 hour.
4. Decant the contents from the microwells into a discard basin. Wash the microwells by filling each with Tris-Tween wash buffer, then decanting the wash buffer into a discard basin. Repeat for a total of 5 washes.
5. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
6. Determine the required volume of rabbit anti-mSAP antibody (1mL/strip or 120µL/well) to prepare. Dilute stock of rabbit anti-mSAP antibody (100X) to working concentration (1X) with Tris-Tween wash buffer.
7. Add 100µL of rabbit anti-mSAP antibody to each well. Incubate at room temperature for 30 minutes. Cover to avoid direct light.
8. Repeat steps 4 and 5.
9. Determine the required volume of HRP conjugated goat anti-rabbit Fc antibody (1mL/strip or 120µL/well) to prepare. Dilute stock of HRP conjugated goat anti-rabbit Fc antibody (100X) to working concentration (1X) with Tris-Tween wash buffer.

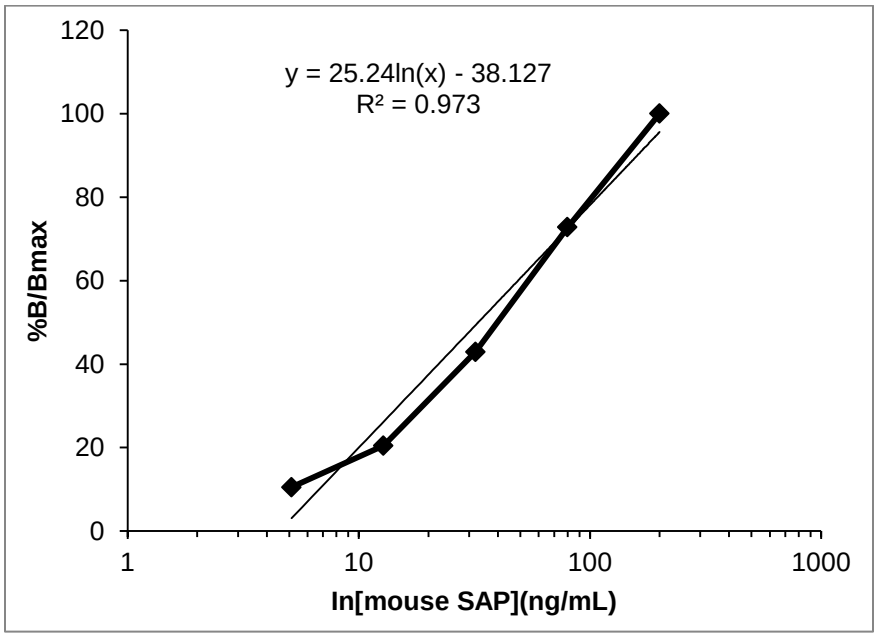
10. Add 100µL of HRP conjugated goat anti-rabbit Fc antibody to each well. Incubate at room temperature for 20 minutes. Cover to avoid direct light.
11. Repeat steps 4 and 5.
12. Add 100µL of Substrate Reagent to each well. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
13. Add 100µL of Stop Solution to each well in the same sequence and at the same pace as the Substrate Reagent was added.
14. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.

INTERPRETATION OF RESULTS

Construct a standard curve using the %B/Bmax values against the concentration of the standards. Unknowns are measured by interpolation from the standard curve. Final concentration must be multiplied by the dilution factor at which samples were prepared to get the actual concentration (ng/mL). If a sample contains mSAP at a greater concentration than the highest standard, it should be diluted appropriately in Tris-Tween wash buffer and re-tested. The extra dilution step should be taken into account when expressing the final result.

ASSAY CHARACTERISTICS

Below is an example of a typical standard curve achieved using the HELICA Mouse SAP Assay.



Intra-assay reproducibility

A typical example of the HELICA Mouse SAP Assay run in 10 replicates yielded the following within assay variation.

Standards (ng/mL)	%B/Bmax	%CV
0.0	1.4	5
5.12	7.9	3
12.8	16.2	5
32.0	32.2	7
80.0	65.9	4
200.0	100.0	3

Inter-assay reproducibility
The HELICA Mouse SAP Assay exhibited the following variability with three different kit lots performed by three operators over a 6 month period.

Standard (ng/mL)	%B/Bmax	%CV
0.0	3.0	35
5.12	10.5	18
12.8	20.5	17
32.0	42.9	10
80.0	72.8	6
200.0	100.0	0

Limitations
Lipemic sera may interfere with specific antibody reaction.

Limit of Detection
The lower and upper limit of quantitation is 5.12 and 200ng/mL, respectively.

Quality Control
It is recommended to routinely run at least two controls, each giving values at the top or bottom regions of the standard curve. An occasional prozone may be encountered in sera with high SAP values. In this situation, due to antigen excess, all the SAP available may not have reacted with the conjugate. Therefore, test at higher dilution(s) to obtain more accurate results.

Cross-Reactivity
Given the extensive homology between mouse SAP and mouse CRP, the cross-reactivity of mouse CRP was tested on the mouse SAP ELISA and shown to be negative.

Antigen	% Cross-reactivity
Mouse CRP	<0.05%

Cross-reactivity for other proteins or SAP from other species has not been tested.

References

1. Mortensen, R.F., Beisel, K., Zeleznik, N.J., and Le, P.T. (1983) Strain dependence of serum amyloid P-component levels and response to inflammation. J Immunol 130(2):885-889.
2. Hind, C.R.K, Collins, P.M., Renn, D., Cook, R.B., Caspi, D., Baltz, M.L., and Pepys, M.B. (1984) Binding specificity of serum amyloid P component for the pyruvate acetal of galactose. J Exp Med 159(4):1058-1069.