



AssayMax™
Mouse Serum Amyloid P Component
ELISA Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 μ l of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 μ l of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 μ l of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 μ l of Chromogen Substrate per well.
Incubate 10 minutes.

Step 5. Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key



Consult instructions for use.

Mouse Serum Amyloid P Component ELISA Kit

Catalog No. EMA8201-1

Sample insert for reference use only

Introduction

Serum amyloid P component (SAP, Serum AP, APCS), a 25kDa pentameric protein, is a normal plasma protein and a universal non-fibrillar constituent of amyloid deposits (1). SAP is a pentraxin similar to C-reactive protein (2, 3).

Principle of the Assay

The AssayMax Mouse Serum Amyloid P Component ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of mouse SAP in **plasma, serum, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures mouse SAP in less than 4 hours. A polyclonal antibody specific for mouse SAP has been pre-coated onto a 96-well microplate with removable strips. SAP in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for mouse SAP, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Mouse Serum Amyloid P Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse SAP.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Mouse Serum Amyloid P Standard:** Mouse SAP in a buffered protein base (32 ng, lyophilized).
- **Biotinylated Mouse Serum Amyloid P Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against mouse SAP (140 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:6000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:6000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes at 4°C to remove debris and assay. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 32 ng of Mouse Serum Amyloid P Standard with 1 ml of MIX Diluent to generate a 32 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (32 ng/ml) 1:2 with MIX Diluent to produce 16, 8, 4, 2, 1, and 0.5 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Mouse SAP] (ng/ml)
P1	1 part Standard (32 ng/ml)	32.0
P2	1 part P1 + 1 part MIX Diluent	16.0
P3	1 part P2 + 1 part MIX Diluent	8.00
P4	1 part P3 + 1 part MIX Diluent	4.00
P5	1 part P4 + 1 part MIX Diluent	2.00
P6	1 part P5 + 1 part MIX Diluent	1.00
P7	1 part P6 + 1 part MIX Diluent	0.50
P8	MIX Diluent	0.00

- **Biotinylated Mouse Serum Amyloid P Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Mouse Serum Amyloid P Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Mouse Serum Amyloid P Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 10 minutes or till the optimal blue color density develop. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some

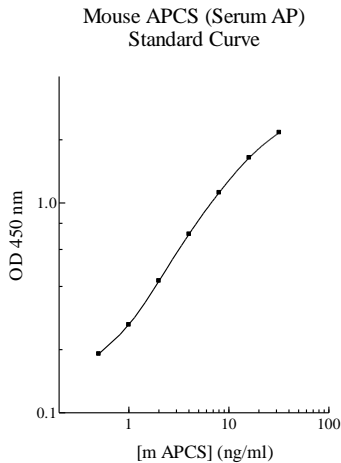
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is used for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of mouse SAP is typically ~ 0.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.0% respectively.

Linearity

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:2000	88%	87%
1:6000	98%	99%
1:8000	107%	104%

Recovery

Standard Added Value	1.0 – 20 ng/ml
Recovery %	84 – 110%
Average Recovery %	97%

Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Mouse	100%
Rat	None
Swine	None
Rabbit	None
Human	None

- 10% FBS in culture media will not affect the assay.

References

- (1) Cathcart ES, Shirahama T, Cohen AS (1967). *Biochim. Biophys. Acta.* 147: 392–393
- (2) Jenny NS, *et al* (2007). *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2007;27:352
- (3) Koenig W. (2007) *Arterioscler Thromb Vasc Biol* 2007 27: 698-700

Version 1.1R

Related Products

- EA8201-1 AssayMax Human Serum Amyloid P Component ELISA Kit (Plasma, Serum, Urine, CSF, and Cell Culture samples)
- ERA8201-1 AssayMax Rat Serum Amyloid P Component ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)

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