User Guide

Mouse SAA-3 ELISA Kit

96-Well Plate

EZMSAA3-12K

Intended Use

This kit is used for the non-radioactive quantification of Mouse SAA-3 in serum, plasma and other biological media. One kit is sufficient to measure 38 unknown samples in duplicate. For Research Use Only. Not for Use in Diagnostic Procedures.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of Mouse SAA-3 from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-Mouse SAA-3 polyclonal antibody,
- Wash away of unbound materials from samples,
- Binding of a biotinylated anti-Mouse SAA-3 polyclonal antibody to the captured Mouse SAA-3,
- Wash away of unbound materials from samples,
- Conjugation of horseradish peroxidase to the immobilized biotinylated antibodies,
- Wash away of free enzyme conjugates, and
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine.

The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured Mouse SAA-3 in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Mouse SAA-3.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C

Reagents Supplied	Volume	Quantity	Cat. No.
Mouse SAA-3 ELISA Plate with 2 plate sealers **Note:** Unused strips should be resealed in the foil pouch with the desiccant provided.	-	1 plate 2 sealers	EP12
10X HRP Wash Buffer Concentrate 10X concentrate of 50 mM Tris Buffered Saline containing Tween®-20.	50 mL	2 bottles	EWB-HRP
Mouse SAA-3 Standard Purified Recombinant GST-tagged Mouse SAA-3	0.25 mL Lyophilized	1 vial	E8012-K
Mouse SAA-3 Quality Controls 1 and 2	0.25 mL/vial	1 vial each	E6012-K
Assay Buffer 0.05 M PBS, pH 7.4, containing 0.025 M EDTA, Sodium azide, 1% BSA and 0.05% Triton™ X-100	10 mL	2 bottles	EABTR
Mouse SAA-3 Detection Antibody	11 mL	1 bottle	E1012
Enzyme Solution	12 mL	1 vial	EHRP
Substrate Solution 3,3′,5,5′-tetramethylbenzidine in buffer (light-sensitive, avoid unnecessary exposure to light)	12 mL	1 bottle	ESS-TMB
Stop Solution 0.3 M HCl **Caution:** Corrosive Solution	12 mL	1 bottle	ET-TMB

Storage and Stability

Recommended storage for kit components is 2-8 °C.

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze thaws should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Reagent Precautions

Sodium Azide

Sodium azide has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Hydrochloric Acid

Hydrochloric acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

Ingredient	Cat. No.	Full Label	
Mouse SAA-3 Detection Antibodies	E1012		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Mouse SAA-3 Quality Controls 1 and 2	E6012-K	 	**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention.
Mouse SAA-3 Standard	E8012-K	 	**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention.
Stop Solution	ET-TMB		**Warning.** May be corrosive to metals
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

Sample Collection and Storage

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ± 2 °C.
3. Transfer and store serum samples in separate tubes. Date and identify each sample.
4. Avoid multiple (> 5) freeze/thaw cycles.
5. To prepare plasma samples, whole blood should be collected into EDTA-plasma tubes and centrifuged immediately after collection. Observe same precautions in the preparation of serum samples.
6. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
7. Avoid using samples with gross hemolysis or lipemia.

Reagent Preparation

Mouse SAA-3 Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Mouse SAA-3 Standard with 0.25 mL distilled or deionized water to give a concentration described in the analysis sheet. Invert and mix gently, let sit for 5 minutes then vortex gently.

2. Label six tubes 1, 2, 3, 4, 5, and 6. Add 0.1 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 0.1 mL of the reconstituted standard to Tube 1, mix well and transfer 0.1 mL of Tube 1 to Tube 2, mix well and transfer 0.1 mL of Tube 2 to Tube 3, mix well and transfer 0.1 mL of Tube 3 to Tube 4, mix well and transfer 0.1 mL of Tube 4 to Tube 5, mix well and transfer 0.1 mL of Tube 5 to Tube 6 and mix well.

   Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of reconstituted standard should be stored at ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard	0.25 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (µg/mL)
Tube 1	0.1 mL	0.1 mL of reconstituted standard	X/2
Tube 2	0.1 mL	0.1 mL of Tube 1	X/4
Tube 3	0.1 mL	0.1 mL of Tube 2	X/8
Tube 4	0.1 mL	0.1 mL of Tube 3	X/16
Tube 5	0.1 mL	0.1 mL of Tube 4	X/32
Tube 6	0.1 mL	0.1 mL of Tube 5	X/64

Mouse SAA-3 Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Mouse SAA-3 Quality Control 1 and Quality Control 2 with 0.25 mL distilled or deionized water into the vials. Invert and mix gently, let sit for 5 minutes then mix well.

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X Wash Buffer concentrate 10-fold by mixing the entire content of each bottle of Wash Buffer with 450 mL deionized water (dilute both bottles with 900 mL deionized water).
2. Remove required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8 °C. Assemble strips in an empty plate holder and add 300 µL of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.
3. Add 90 µL Assay Buffer into all wells.
4. Add in duplicate 10 µL Assay Buffer to blank wells. (Refer to Assay Procedure for suggested well Orientations.)
5. Add in duplicate 10 µL Mouse SAA-3 Standards in order of ascending concentration to the appropriate wells. Add in duplicate 10 µL QC1 and 10 µL QC2 to the appropriate wells. Add sequentially 10 µL of samples in duplicate to the remaining wells. For best results all additions should be completed within 30 minutes.
6. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.
7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
8. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
9. Add 100 µL Detection Antibody to each well. Cover the plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.
10. Remove sealer and decant solution from the plate. Tap as before to remove residual solutions in the wells.
11. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

12. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

13. Remove sealer, decant solution from the plate, and tap plate to remove the residual fluid.

14. Wash wells 6 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

15. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for 5-20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of SAA-3 standards with intensity proportional to increasing concentrations of SAA-3.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

16. Remove sealer and add 100 µL of Stop Solution (**Caution:** Corrosive solution) and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference in absorbance units. The absorbance of the highest SAA-3 standard should be approximately 1.5–2.2, or not to exceed the capability of the plate reader used.

Assay Procedure for Mouse SAA-3 ELISA Kit

Well #	Step 1	Step 2	Step 3-4	Step 5	Step 6-8	Step 9	Step 9-11	Step 12	Step 12-14	Step 15-16		
			Assay Buffer	Standards/ QCs/Samples		Detection Antibody		Enzyme Solution		Substrate		Stop Solution
A1, B1	Dilute 2 bottles of 10X Wash Buffer with 900mL Deionized Water.	Add 300 µL Wash Buffer to each well and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels	100 µL	--	Seal, Agitate, Incubate 2 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer	100 µL	Seal, Agitate, Incubate 1 hour at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels. Wash 3X with 300 µL Wash Buffer	100 µL	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 5-20 minutes at Room Temperature.	100 µL	Read Absorbance at 450 nm and 590 nm.
C1, D1			90 µL	10 µL of Standard #6								
E1, F1				10 µL of Standard #5								
G1, H1				10 µL of Standard #4								
A2, B2				10 µL of Standard #3								
C2, D2				10 µL of Standard #2								
E2, F2				10 µL of Standard #1								
G2, H2				10 µL of Original Standard								
A3, B3				10 µL of QC 1								
C3, D3				10 µL of QC 2								
E3, F3				10 µL of Sample								
G3, H3				10 µL of Sample								
				10 µL of Sample								

Microtiter Plate Arrangement

Mouse SAA-3 ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 3 Standard	QC 1	Etc.								
B	Blank	Tube 3 Standard	QC 1									
C	Tube 6 Standard	Tube 2 Standard	QC 2									
D	Tube 6 Standard	Tube 2 Standard	QC 2									
E	Tube 5 Standard	Tube 1 Standard	Sample 1									
F	Tube 5 Standard	Tube 1 Standard	Sample 1									
G	Tube 4 Standard	Original Standard	Sample 2									
H	Tube 4 Standard	Original Standard	Sample 2									

Calculations

The dose-response curve of this assay fits best to a 4 or 5-parameter logistic equation. The results of unknown samples using GST-tagged Mouse SAA-3 as standard can be calculated with any computer program having a 4 or 5-parameter logistic function.

Note: When sample volumes assayed differ from 10 µL (in normal assay), an appropriate mathematical adjustment must be made to accommodate for additional dilution factor (for example, if 5 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µL, compensate the volume deficit with assay buffer.

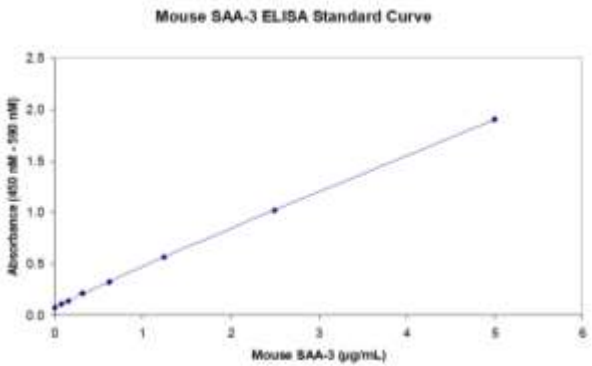
Interpretation

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is > 10% CV, repeat the sample.
3. The limit of sensitivity of this assay is 0.078 µg/mL Mouse SAA-3 (10 µL sample size).
4. The appropriate range of this assay is 0.078 to 5 µg/mL Mouse SAA-3 (20 µL sample size). Any result greater than 5 µg/mL in a 10 µL sample assayed should be diluted and repeated using assay buffer as diluent until it falls within range.

Normal Range

SAA-3 levels in "normal" CD-1 mouse range from 0.1-1.0 µg/mL.

Standard Curve



Typical Standard Curve - Not to be used to calculate data

Assay Characteristics

Sensitivity

The lowest level of Mouse SAA-3 standard used in this assay is 0.078 µg/mL (10 µL sample size).

Specificity

Mouse SAA-3	100%
Complement C1q C1740 10 µg/mL	N.D.
Rat Tail Collagen Type I C7661 10 µg/mL	N.D.
Hu Placenta Collagen Type VI C7521 10 µg/mL	N.D.
mPTX-3 0.5 µg/mL	N.D.
24P3 2.5 µg/mL	N.D.
mAGP 1.0 µg/mL	N.D.
mAdipsin 2.5 µg/mL	N.D.
GST protein 1 µg/mL	N.D.
Complement C1q C1740 10 µg/mL	N.D.
Rat Tail Collagen Type | C7661 10 µg/mL	N.D.

N.D.: Not detectable

Precision

Intra-Assay Variation

	Mean SAA-3 Levels (ng/mL)	Intra-Assay %CV
1	0.2	14%
2	1.8	3%

Inter-Assay Variation

	Mean SAA-3 Levels (ng/mL)	Inter-Assay %CV
1	0.2	13%
2	1.8	10%

The assay variations of Mouse SAA-3 ELISA kits were studied on two samples with varying concentrations of exogenous SAA-3. Intra-assay variation was calculated from eight determinations from a single assay. Inter-assay variation was calculated from single determinations in duplicate from seven separate assays.

Spike and Recovery

Exogenous Mouse SAA-3	% Expected (n = 5)
0.313 µg/mL	97.± 0 30
0.625 µg/mL	96 ± 23
1.250 µg/mL	101 ± 19

Three serum and two plasma samples were spiked with different amounts of exogenous Mouse SAA-3. These spiked serum and plasma samples were assayed by Mouse SAA-3 ELISA. Expected values are the basal levels plus the spiked amount (0.313, 0.625 and 1.25 µg/mL) of Mouse SAA-3. The % Expected is observed value divided by expected value X 100 (Mean ± SD).

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- Do not let the absorbency reading of the highest standard reach 2.0 units or higher before adding the stop solution.
- High absorbance in background or blank wells could be due to:
    - Well cross contamination by standard solution or sample and
    - Inadequate washing of wells with HRP.

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Catalogue Number
Mouse SAA-3 ELISA Plate	EP12
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Mouse SAA-3 ELISA Standard	E8012-K
Quality Controls 1 & 2	E6012-K
Assay Buffer (10 mL/vial)	EABTR
Enzyme Solution (12 mL/vial)	EHRP
Mouse SAA-3 Detection Antibody (11 mL/vial)	E1012
Substrate (12 mL)	ESS-TMB
Stop Solution (12 mL/vial)	ET-TMB

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.