SAH ELISA Kit

SAH ELISA Kit is an Enzyme Immunoassay kit for the quantification of SAH in plasma, serum, tissue homogenate, cell lysate or other biological fluids.

Catalog number: ARG83602

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

S-adenosylmethionine (SAM) is a methyl donor involved in the transfer of a methyl group to DNA, proteins, phospholipids, RNA, and neurotransmitters. Reactions that break down and regenerate SAM have been named the SAM cycle. SAM-dependent methylases use SAM as a substrate to yield S-adenosylhomocysteine (SAH), which is further broken down to homocysteine and adenosine by s-adenosylhomocysteine hydrolase. The homocysteine can be regenerated to methionine and finally SAM by methionine synthases

Donation of the SAM methyl group converts SAM into SAH, the latter being a potent inhibitor of methylation. For this reason, the SAM/SAH ratio has been used as an index of methylation potential in a cell. SAH in plasma has been found to be a more sensitive indicator for vascular disease risk than homocysteine. In addition, distinct classes of riboswitches that recognize SAH but not SAM and regulate gene expression have been discovered.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. After coated SAH Conjugate onto a microtiter plate, SAH of a sample competes with a SAH-antibody for binding to the coated Conjugate. After incubation the unbound antibody is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of SAH in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of SAH. SAH concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard and 1000X SAH Conjugate at -80°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
1000X SAH Conjugate	1 vial (20 µL)	-80°C
Standard	1 vial (20 µL; 1.25 mM)	-80°C
500X SAH Antibody	1 vial (15 µL)	4°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
Diluent Buffer	50 mL	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)
- 1X PBS

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard and 1000X SAH Conjugate at -80°C. Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at-80ºC for up to three months. Normal serum samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.

Plasma- Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at-80ºC for up to three months. Normal plasma samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.

Tissue homogenate- Weigh and homogenize the tissue on ice in 5-10 mL cold PBS per gram of tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Store any unused supernatant at-80°C for up to three months.

Cell lysate- Collect cells by centrifuging at 2000 x g for 10 minutes at 4°C. Sonicate or homogenize the cell pellet on ice in 1-2 mL cold PBS. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Aliquot and store the supernatant for use in the assay. Store any unused supernatant at-80°C for up to three months.

Other biological fluids- Centrifuge samples for 10 minutes at 1000 g at 4ºC and recover supernatant. Assay immediately or store samples at -80ºC for up to three months.

REAGENT PREPARATION

- 1X SAH Conjugate: Dilute immediately before use; dilute 1000X SAH Conjugate into 1X PBS to yield 1X SAH Conjugate.
- SAH Conjugate Coated Plate: Add 100 µL 1X SAH conjugate coating solution to each well and incubate overnight at 4°C. After incubation, remove the diluted SAH conjugate, blotting plate on paper towels to remove excess fluid. Wash wells 3 times with 200 µL of PBS and blot on paper towels to remove excess fluid. Add 200 µL of Diluent Buffer to each well and block for 1 hour at room temperature. Transfer the plate to 4ºC until ready to begin the assay.

  Note: The SAH Conjugate Coated Plate is not stable long-term. We recommend using it within 24 hours after coating.
- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer.
- 1X SAH Antibody: Dilute immediately before use; dilute 500X SAH Antibody into Diluent Buffer to yield 1X SAH Antibody.
- 1X HRP-Streptavidin Solution: Dilute immediately before use; dilute 1000X HRP-Streptavidin Solution into Diluent Buffer to yield 1X HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- Standard: Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. The Diluent

Buffer serves as zero standard (0 µM), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	SAH (µM)	Diluent Buffer(µL)	Standar(µL)
S1	12.5	495	5 (1.25 mM Stock)
S2	6.25	150	150 of S1
S3	3.125	150	150 of S2
S4	1.563	150	150 of S3
S5	0.781	150	150 of S4
S6	0.391	150	150 of S5
S7	0.195	150	150 of S6
S0	0	150	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Remove the Diluent Buffer from the plate, and add **50 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **10 min** at **RT**.
3. Add **50 µL** of **1X SAH antibody** into each wells.
4. Cover the plate and incubate for **1 hour** at **RT**.
5. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 100 µL of 1X HRP-Streptavidin Solution to each well.
7. Cover the plate and incubate for 1 hour at room temperature in the dark.
8. Aspirate each well and wash plate as step 5.
9. Add 100 µL of TMB Substrate in each well.
10. Incubate for 2-30 mins at room temperature in the dark.
11. Add 100 µL of Stop Solution to each well to stop the reaction.
12. Read the absorbance with a plate reader at O.D. 450 nm. It is recommended reading the absorbance within 10 minutes after adding the stop solution.

    Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

CALCULATION OF RESULTS

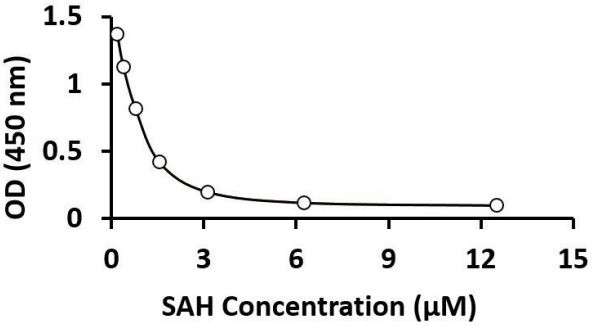
1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated

automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the SAH ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.1 µM

Assay Range

0.195- 12.5 µM