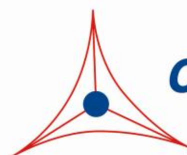
Product Manual

S-Adenosylmethionine (SAM) ELISA Kit

Catalog Number

MET-5152 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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 (Figure 1). 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. Patients with coronary artery disease have been shown to have lower whole blood SAM levels compared to normal individuals. Endothelium dependent vasodilation has been correlated with increased levels of SAM. Additionally, high SAM levels were associated with decreases in carotid intima media thickness in non-diabetic individuals.

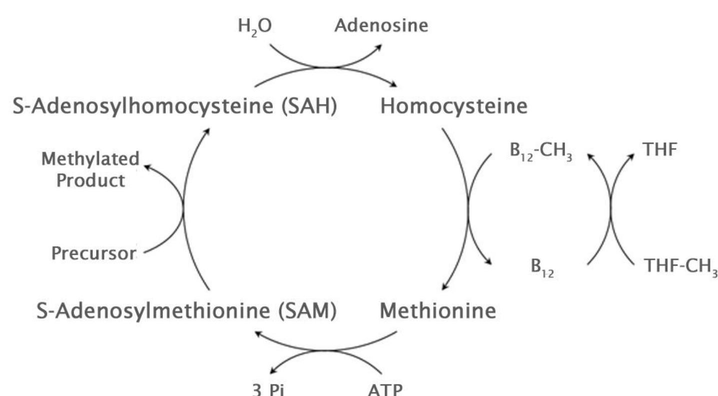


Figure 1. The SAM cycle.

Cell Biolabs' SAM ELISA Kit is a competitive enzyme immunoassay developed for the detection and quantitation of SAM in plasma, serum, lysates, or other biological fluid samples. The kit has a detection sensitivity limit of 0.625 nM free SAM. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. MET-5151-C: S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine ELISA Combo Kit
2. MET-5090: Adenosine Assay Kit (Fluorometric)
3. MET-5158: Methionine Assay Kit (Fluorometric)
4. MET-5163: ATP Assay Kit (Fluorometric)
5. MET-5125: Pyruvate Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. 96 Well Protein Binding Plate (Part No. 231001): One strip well 96 well plate.
2. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
3. Assay Diluent (Part No. 310804): One 50 mL bottle.

4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
5. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
6. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on dry ice)

1. SAM Conjugate (100X) (Part No. 267201): One 100 µL vial.
2. SAM Standard (Part No. 51521D): One 40 µL vial of a stabilized form of free s-adenosylmethionine at 1.2 µM.
3. Anti-SAM Antibody (500X) (Part No. 267202): One 15 µL vial.

Materials Not Supplied

1. Phosphate Buffered Saline (PBS)
2. PBS containing 0.1% Bovine Serum Albumin (BSA)

Storage

Upon receipt, store SAM Conjugate and SAM Standard at -80ºC. Store the rest of the kit at 4ºC.

Preparation of Reagents

- SAM Conjugate Coated Plate: Determine the number of wells to be used, and dilute the SAM Conjugate 1:100 in PBS. Add 100 µL of 1X SAM conjugate to each well of the 96-well Protein Binding Plate. Incubate overnight at 4ºC. Remove the diluted SAM 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 Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4ºC until ready to begin the assay.

Note: The SAM Conjugate Coated Plate is not stable long-term. We recommend using it within 24 hours after coating.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-SAM Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the Anti-SAM Antibody 1:500 and the Secondary Antibody HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of SAM standards in the concentration range of 0 to 40 nM in Assay Diluent (Table 1).

Standard Tubes	1.2 µM SAM Standard (µL)	Assay Diluent (µL)	SAM (nM)
1	10	290	40
2	150 of Tube #1	150	20
3	150 of Tube #2	150	10
4	150 of Tube #3	150	5
5	150 of Tube #4	150	2.5
6	150 of Tube #5	150	1.25
7	150 of Tube #6	150	0.625
8	0	150	0

Table 1. Preparation of SAM Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- 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.
- 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.
- 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.

Assay Protocol

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.

1. Prepare and mix all reagents thoroughly before use.
2. Each unknown sample (see Preparation of Samples section), SAM standard, and blank should be assayed in duplicate.
3. Remove the Assay Diluent from the plate and add 50 µL of unknown sample or standard to the SAM Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-SAM Antibody (see Preparation of Reagents section) to each tested well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After each wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted Secondary Antibody HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.
7. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.
8. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical SAM ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

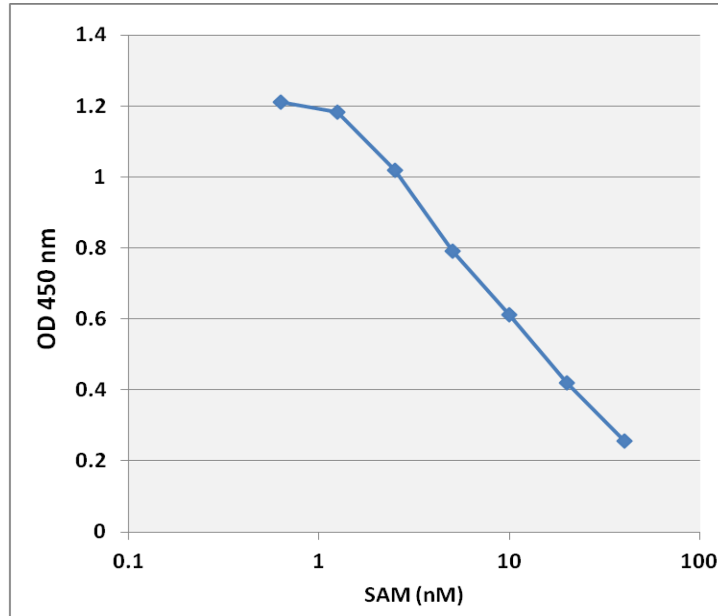


Figure 2: SAM Standard Curve.

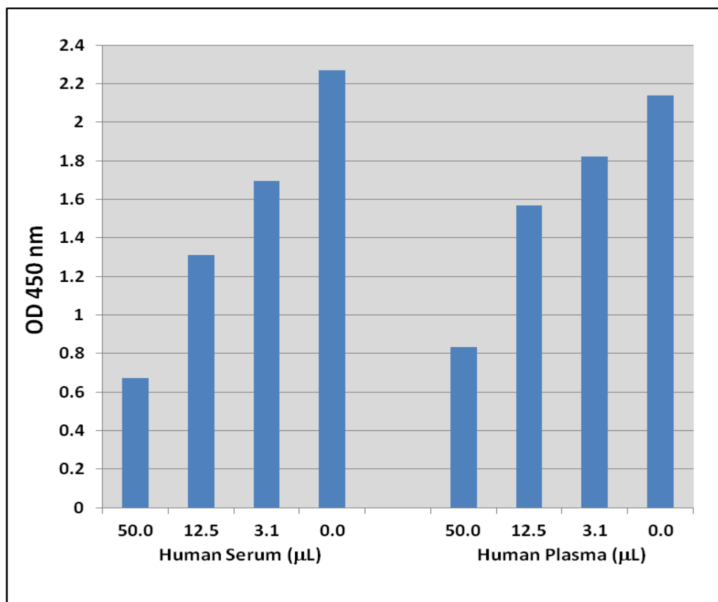


Figure 3: SAM Detection in Human Serum and Plasma.

References

1. Hirsch S, Ronco AM, Guerrero-Bosagna C, de la Maza MP, Liva L, Barrera G, Llanos M, Alliende MA, Silva F, and Bunout D (2008) *Nutrition.* **11-12**:1103-1109.

2. Pey AL, Majtan T, Sanchez-Ruiz JM, and Kraus JP (2013) *Biochem J.* **449**: 109-121
3. Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, and James SJ (2000) J. *Biol. Chem*. **275**:29318-29323.
4. Stabler SP and Allen RH (2004) *Clin. Chem*. **50**: 365-372.

Recent Product Citations

1. Beauchamp, L.C. et al. (2025). Evidence of COMT dysfunction in the olfactory bulb in Parkinson's disease. *Acta Neuropathol*. **149**(1):21. doi: 10.1007/s00401-025-02861-y.
2. Malaviya, P. & Kowluru, R.A. (2024). Diabetic Retinopathy and Regulation of Mitochondrial Glutathione-Glutathione Peroxidase Axis in Hyperhomocysteinemia. *Antioxidants (Basel)*. **13**(3):254. doi: 10.3390/antiox13030254.
3. Hagemann, C.A. et al. (2022). A liver secretome gene signature-based approach for determining circulating biomarkers of NAFLD severity. *PLoS One*. **17**(10):e0275901. doi: 10.1371/journal.pone.0275901.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2021-2025: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.