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Product Manual

# S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine (SAH) ELISA Combo Kit

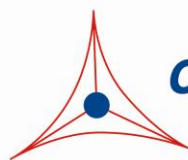
Catalog Number

MET-5151-C

96 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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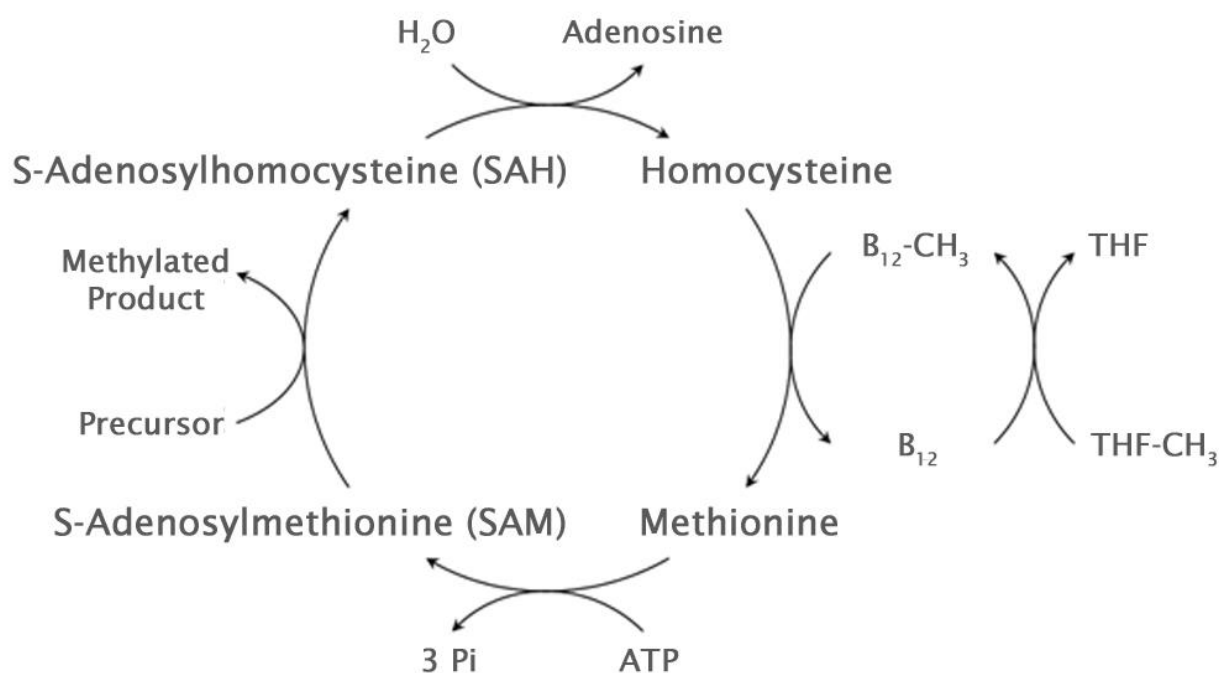
**CELL BIOLABS, INC.**

*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. 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.



**Figure 1. The SAM cycle.**

Cell Biolabs' SAM and SAH Combo ELISA Kit is a competitive enzyme immunoassay developed for the detection and quantitation of SAM and SAH in plasma, serum, lysates, or other biological fluid samples. The kit has a detection sensitivity limit of 0.625 nM for free SAM and 0.2  $\mu$ M for free SAH. Each kit provides sufficient reagents to perform up to 96 total assays for SAM or SAH, including standard curves and unknown samples.

## **Related Products**

1. MET-5090: Adenosine Assay Kit (Fluorometric)
2. MET-5158: Methionine Assay Kit (Fluorometric)

3. MET-5163: ATP Assay Kit (Fluorometric)
4. MET-5019: Total Phosphatidic Acid 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  $\mu$ L 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  $\mu$ L vial.
2. SAH Conjugate (1000X) (Part No. 267101): One 20  $\mu$ L vial.
3. SAM Standard (Part No. 51521D): One 40  $\mu$ L vial of a stabilized form of free s-adenosylmethionine at 1.2  $\mu$ M.
4. SAH Standard (Part No. 51511D): One 20  $\mu$ L vial of 1.25 mM free s-adenosylhomocysteine.
5. Anti-SAM Antibody (500X) (Part No. 267202): One 15  $\mu$ L vial.
6. Anti-SAH Antibody (500X) (Part No. 267102): One 15  $\mu$ L vial.

## **Materials Not Supplied**

1. Plasma, serum, or other biological fluids
2. Phosphate Buffered Saline (PBS)
3. PBS containing 0.1% Bovine Serum Albumin (BSA)

## **Storage**

Upon receipt, store the SAM Conjugate, SAH Conjugate, SAM Standard and SAH Standard at  $-80^{\circ}\text{C}$ . Store the rest of the kit at  $4^{\circ}\text{C}$ .

## **Preparation of Reagents**

- SAM Conjugate or SAH Conjugate Coated Plates: Determine the number of wells to be used, and dilute the SAM Conjugate 1:100 into PBS or dilute the SAH Conjugate 1:1000 into PBS. Add 100  $\mu$ L of 1X conjugate to each well of the 96-well Protein Binding Plate. Incubate overnight at  $4^{\circ}\text{C}$ . Remove the diluted conjugate, blotting plate on paper towels to remove excess fluid. Wash wells 3 times with 200  $\mu$ L of PBS and blot on paper towels to remove excess fluid. Add 200  $\mu$ L of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to  $4^{\circ}\text{C}$  until ready to begin the assay.

*Note: Each 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, Anti-SAH Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the Anti-SAM or Anti-SAH Antibody 1:500 and the Secondary Antibody HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

### **Preparation of Standard Curves**

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 $\mu$ M SAM Standard ( $\mu$ L)	Assay Diluent ( $\mu$ L)	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.**

Prepare a dilution series of SAH standards in the concentration range of 0 to 12.5  $\mu$ M assay diluent (Table 1).

Standard Tubes	1.25 mM SAH Standard ( $\mu$ L)	Assay Diluent ( $\mu$ L)	SAH ( $\mu$ M)
1	5	495	12.5
2	150 of Tube #1	150	6.25
3	150 of Tube #2	150	3.125
4	150 of Tube #3	150	1.563
5	150 of Tube #4	150	0.781
6	150 of Tube #5	150	0.391
7	150 of Tube #6	150	0.195
8	0	150	0

**Table 2. Preparation of SAH 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 4°C. Remove the plasma and assay immediately or store samples at -80°C 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 4°C. Assay immediately or store samples at -80°C 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 4°C and recover supernatant. Assay immediately or store samples at -80°C 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.*

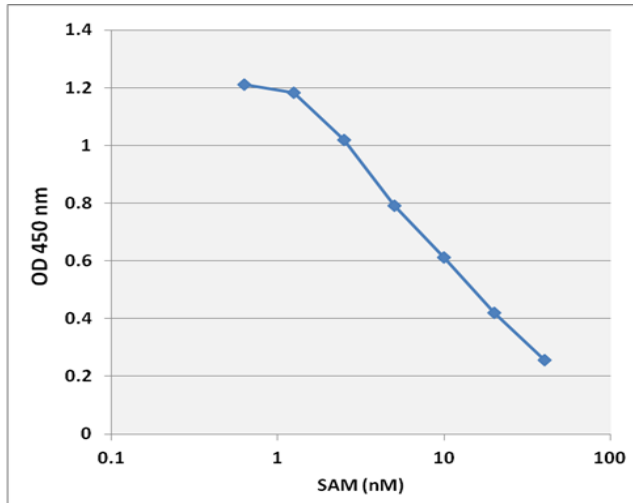
1. Prepare and mix all reagents thoroughly before use.
2. Each unknown sample (see Preparation of Samples section), SAM or SAH standard, and blank should be assayed in duplicate.
3. Remove the Assay Diluent from the plate and add 50 µL of unknown sample or standard to the appropriate Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-SAM or Anti-SAH 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.*

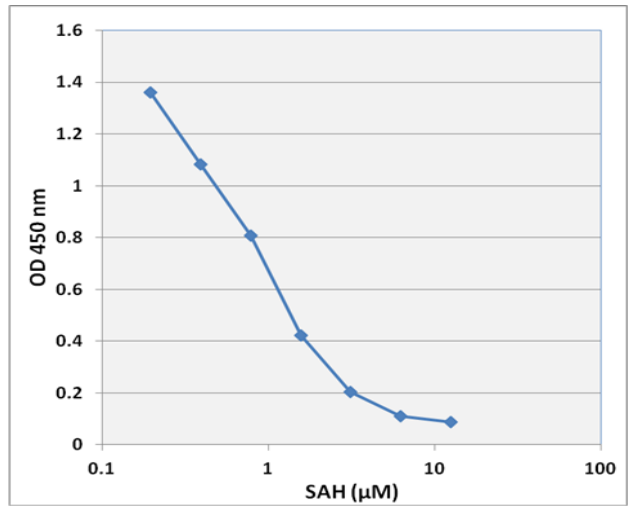
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

### **Example of Results**

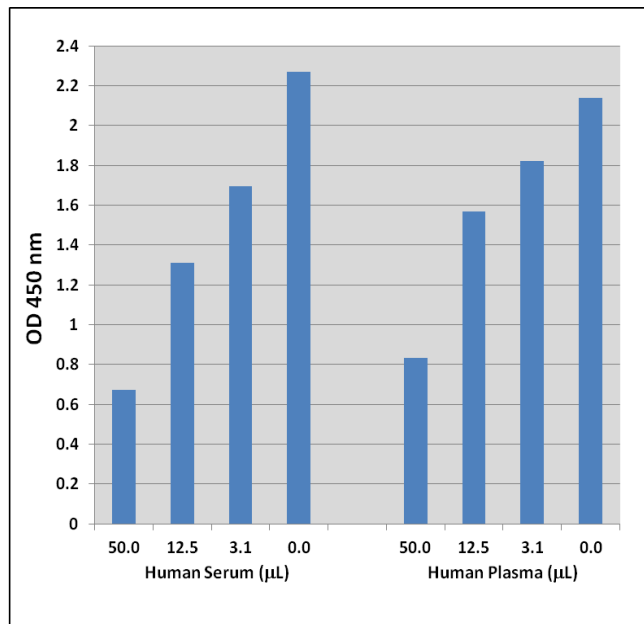
The following figures demonstrate typical SAM and SAH ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.



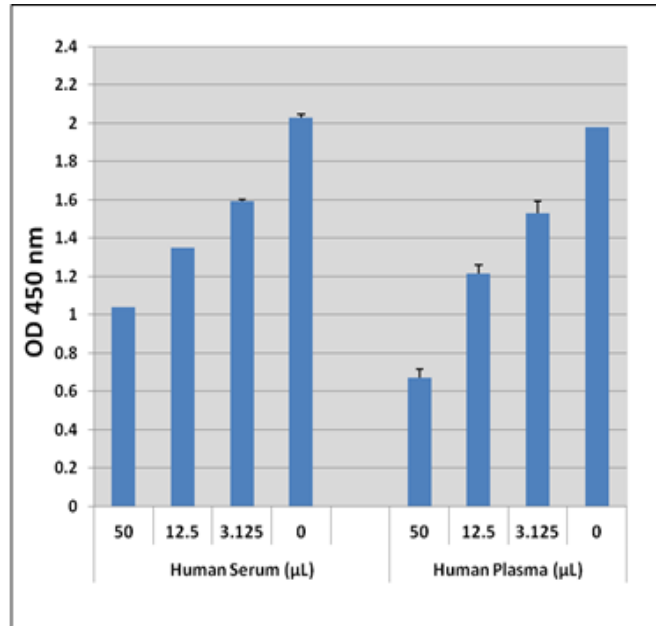
**Figure 2: SAM Standard Curve**



**Figure 3: SAH Standard Curve.**



**Figure 4: SAM Detection in Human Serum and Plasma**



**Figure 5: SAH 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.
5. Wagner C and Koury MJ (2007) *Am J. Clin Nutr.* **86**:1581-1585.
6. Wang JX and Breaker RR (2008) *Biochem Cell Biol.* **86**: 157-168.

### **Recent Product Citations**

1. Kadam, I. et al. (2023). Associations between nutrients in one-carbon metabolism and fetal DNA methylation in pregnancies with or without gestational diabetes mellitus. *Clin Epigenet.* **15**:137. doi: 10.1186/s13148-023-01554-1.
2. Rajabian, N. et al. (2023). Methionine adenosyltransferase2A inhibition restores metabolism to improve regenerative capacity and strength of aged skeletal muscle. *Nat Commun.* **14**(1):886. doi: 10.1038/s41467-023-36483-3.

### **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.

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