S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine (SAH) Related Reagents and their Applications

Sheryl Hao, MD., Ph.D.
Arthus Biosystems
2600 Hilltop Drive
Richmond, CA 94806
Email: sheryl.hao@arthusbio.com
Web: http://www.arthusbio.com

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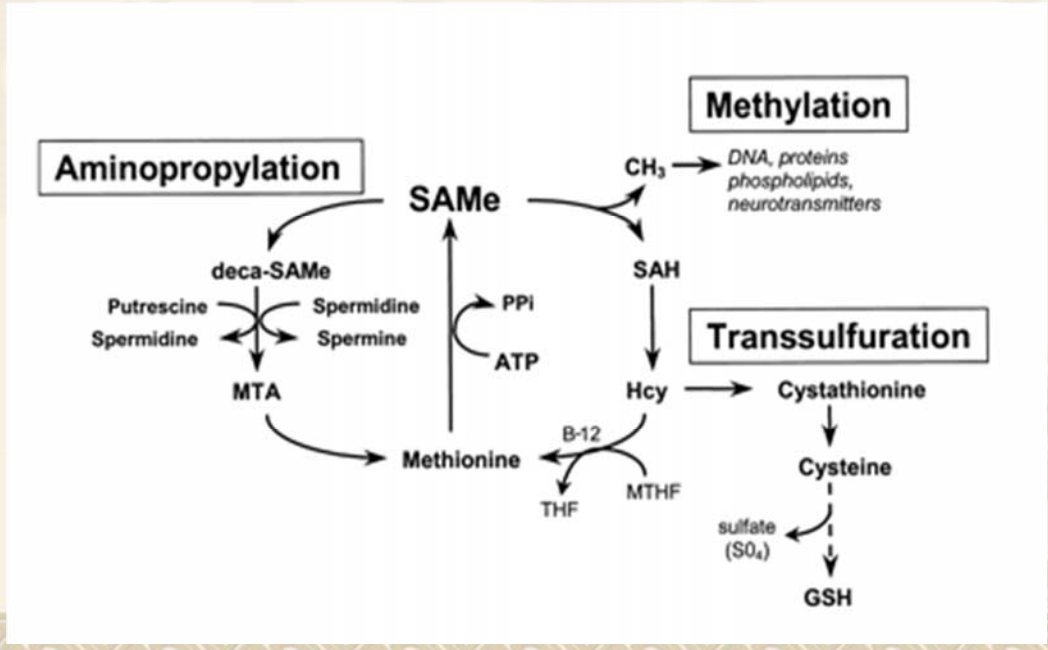
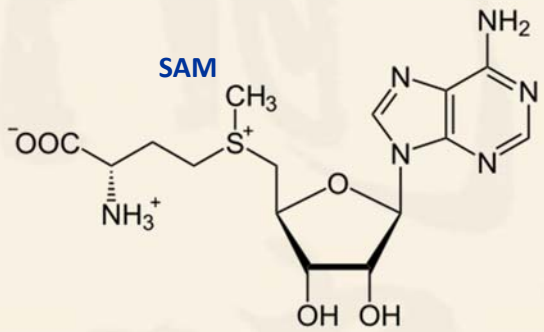
Part 1: Understand S-adenosylmethionine (SAM), S-adenosylhomocysterin (SAH), Methylation Index and Methionine Cycle

S-adenosylmethionine (SAM) is the key molecule in methionine cycle. It plays essential roles in the synthesis and metabolism of nucleic acids, proteins as well as maintaining the mobility of cytoplasmic membranes.

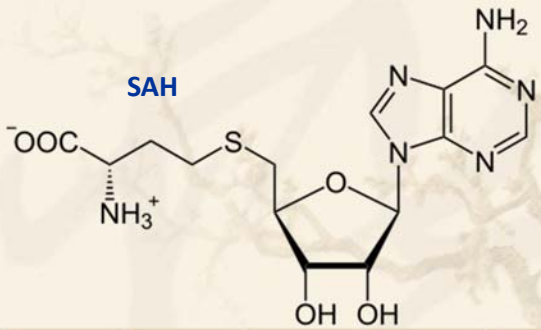


SAM is an intrinsically unstable molecule with MW 398.44, and its optical density maximum of 258-260 nm is not a distinguished absorption, the determination of its concentration in various biological fluids and tissues has always been a challenging task.

SAM



SAH



S-Adenosylmethionine (AdoMet) is metabolized through (a) transfer of its methyl group to a variety of methyl acceptors, (b) decarboxylation followed by aminopropylation leading to polyamine synthesis, and (c) cleavage of the bond between the sulfur atom and carbon 4 of the amino acid chain, resulting in formation of methylthioadenosine and homoserine thiolactone.

About two-thirds of the metabolized compound being utilized via transmethylation and cleavage to methylthioadenosine and one-third via decarboxylation.

A better precursor than methionine both in creatine formation and in the utilization of the sulfur atom in transsulfuration reactions.

Important roles of SAM in birth defects, cardiovascular disease, tumors, liver diseases, mood problems, diabetes, digestive diseases, chronic fatigue, geriatric diseases such as Alzheimer's, Parkinson's Disease, etc.
SAH and homocysteine are closely related to cardiovascular diseases. SAH is considered as a disease marker.

Spermidine serves vital roles in **cell survival**. Spermidine synchronizes an array of biological processes (such as Ca2+, Na+, K+ -ATPase) thus maintaining **membrane** potential and controlling intracellular pH and volume. Spermidine regulates biological processes, such as Ca2+ influx by glutamatergic N-methyl-d-aspartate receptor (NMDA receptor), which has been associated with nitric oxide synthase (NOS) and cGMP/PKG pathway activation and a decrease of Na+,K+-ATPase activity in cerebral cortex synaptosomes.

Spermidine is a **longevity** agent due to its impact on chromatin-mediated regulation of gene expression.

Spermine is a polyamine involved in cellular metabolism found in all eukaryotic cells. The precursor for synthesis of spermine is the amino acid ornithine. It is found in a wide variety of organisms and tissues and is an essential growth factor in some bacteria. It is found as a polycation at physiological pH. Spermine is associated with nucleic acids and is thought to **stabilize helical structure**, in particular, in viruses.

How to measure SAM and SAH?

Since SAM is an intrinsically unstable molecule, the determination of its concentration in various biological fluids and tissues has been challenging.

Currently known: HPLC, LC-MS/MS

Now adding: Immunoassay -- A simple, convenient method that does not require costly instrumentation

Given high quality anti-SAM and anti-SAH antibodies, immunoassays are ideal for:

(1) Sensitive: about 2nM SAM and 15nM SAH with ELISA.
(2) Highly specific: we know what we are looking at.
(3) Quick and convenient
(4) No need of special equipment
(5) Potential for development IVD or POCT products with immunological methods

Why using immunoassays for SAM and SAH?

HPLC is replaced by MS due to its inaccurate, poor sensitivity and hard to separate SAM and SAH.

Limitations and problems of the prevailing LC-MS/MS :

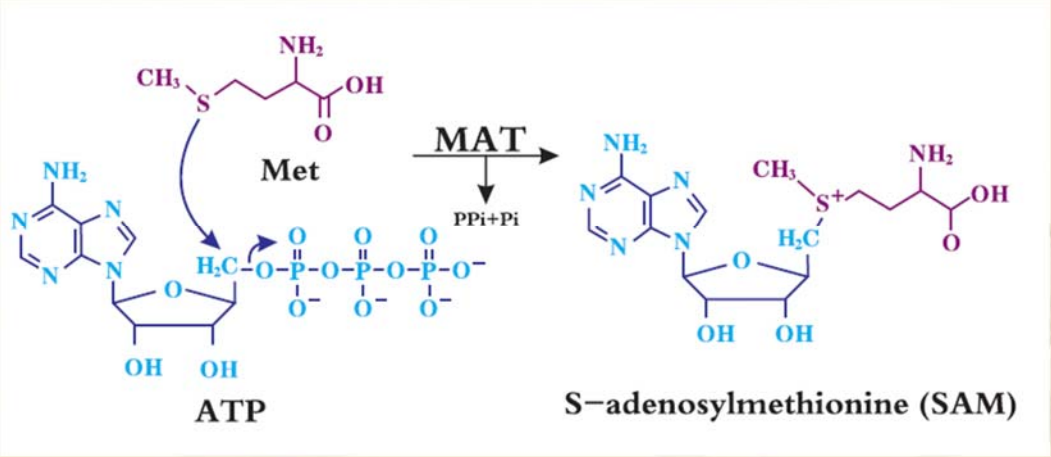
(1) No consideration of biological activity-relevance of the metabolites it detects. It may not be accurate and complete from the biological perspectives.
(2) The chemical methods can only detect free form of SAM or SAH excluding any form of SAM or SAH associated with other bio-molecules.
(3) Just because LC-MS/MS cannot detect from a sample the SAM molecules that falls within that narrow molecular weight specification may not always mean SAM has been totally degraded, disappeared and non-functioning.
(4) The SAM standard used in training LC-MS/MS are not exactly the same as the SAM in living cells, yet in technology like LC-MS/MS, the exact same molecule as the one to be measured is required as the standard.
(5) **Fatal issue**: Doubt of GC-MS and LC-MS analytical methods that may not accurately measure metabolites due to unwanted changes caused by manipulation processes including sample extraction, preprocessing and during the lengthy measurement cycle, which involve high temperature with inorganic solvent that may change the metabolites to be measured. See link below:
http://cen.acs.org/articles/93/i42/Heated-Dispute-Over-Analytical-Method.html

Part 2: Characterization and Uses of

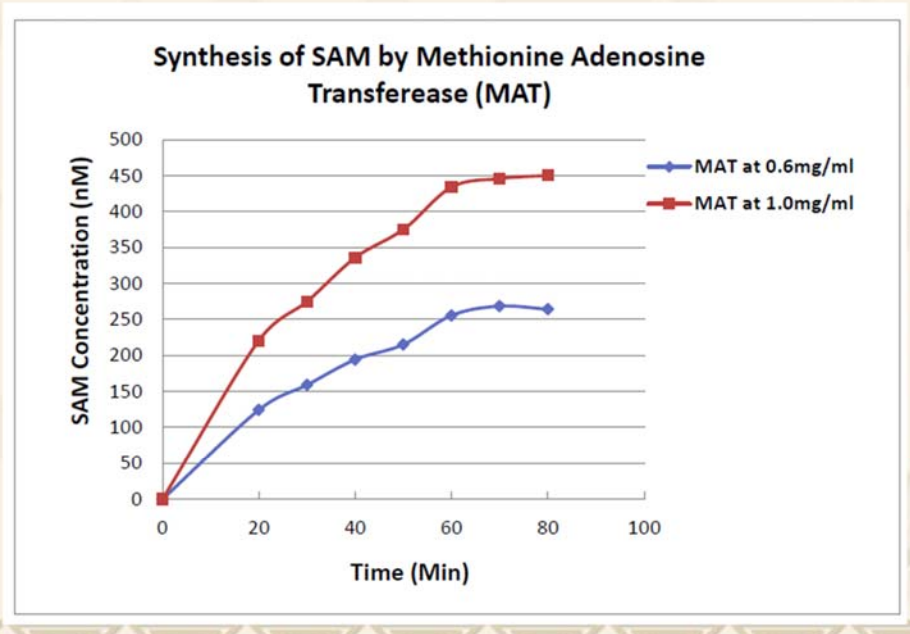
anti-SAM and anti-SAH Antibodies

SAM Biosynthesized from Methionine and ATP

Dose-dependent competition was measured as a sample was added to the cELISA. Any SAM from a sample competes with the coated SAM haptan to bind RP-conjugated anti-SAM antibody. The sample is the product of the following reaction: MAT was added to Met and ATP in an proper buffer at 37°C.



The following has been observed in all anti-SAM antibodies we have. It indicates that all anti-SAM antibodies can specifically binds physiologically produced SAM



Mouse anti-SAM Cross Reactions to Analogs

S-Adenosylmethionine: 100%

S-Adenosylhomocysteine: <1%

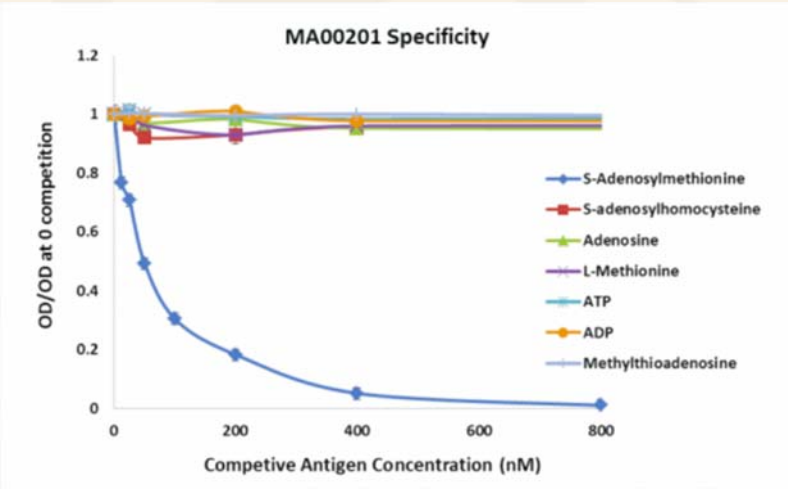
Adenosine: <1%

L-Methionine: <1%

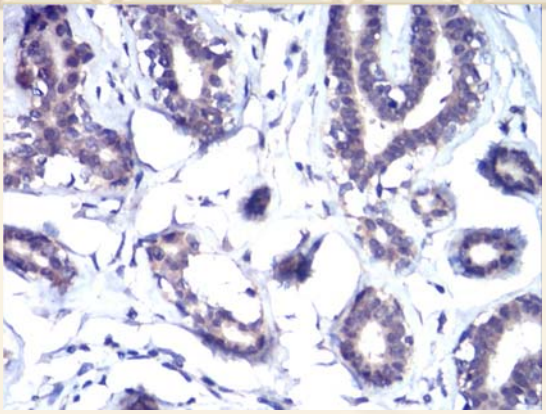
Methythioadenosine (MTA): <1%

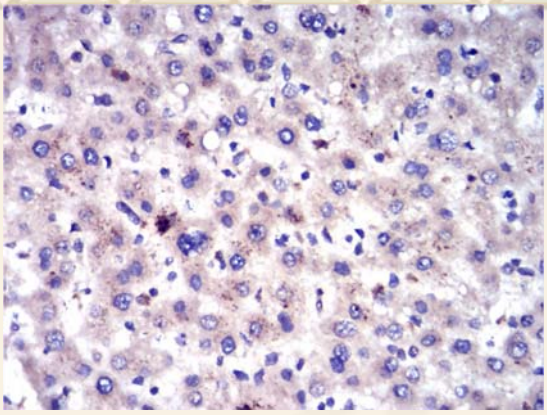
ADP < 1%

ATP < 1%

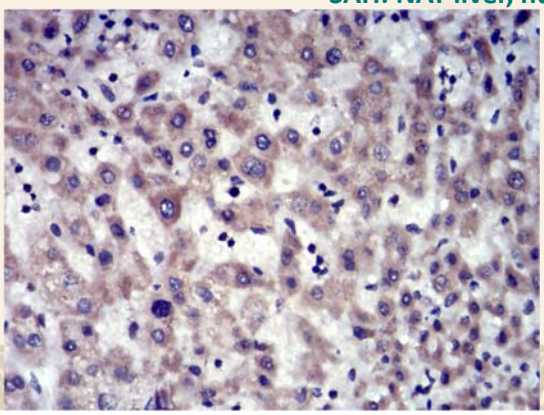


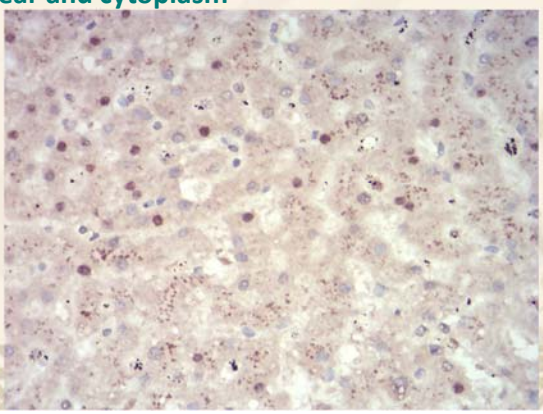
SAM: NAT (Adjacent Normal Tissue) breast and liver, ubiquitous in cytoplasm



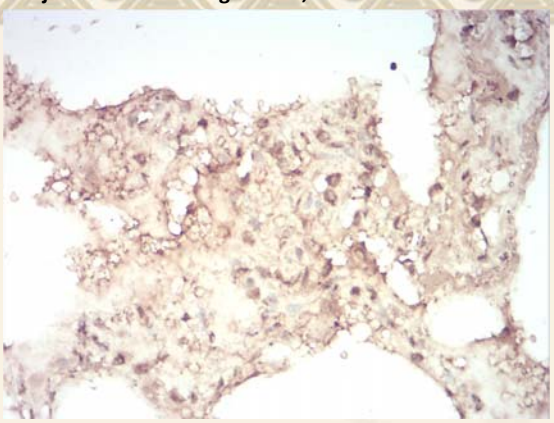


SAH: NAT liver, nuclear and cytoplasm

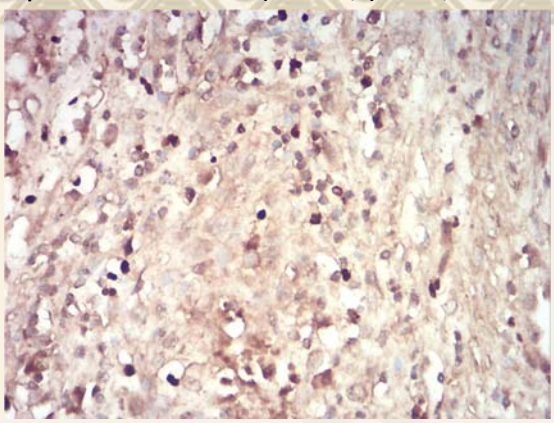




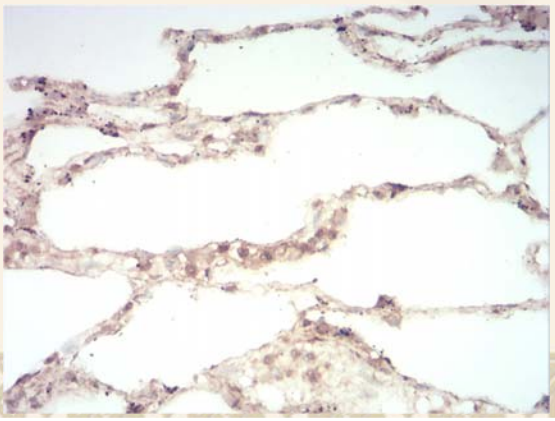
Adjacent normal lung – M56, SAM



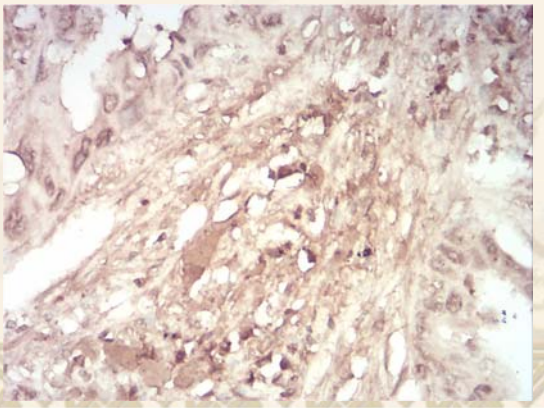
Squamous cell carcinoma (T2N0M0, I) – M56, SAM



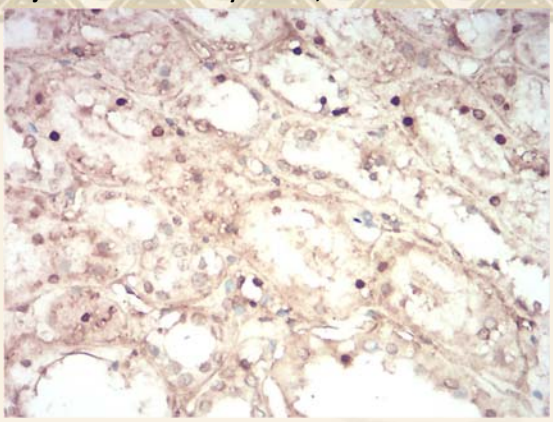
Adjacent normal lung NAT – M39, SAM



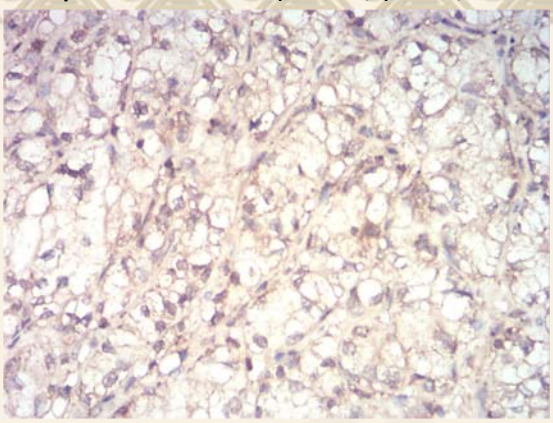
Adenocarcinoma (T2N0M0, II) – M39, SAM



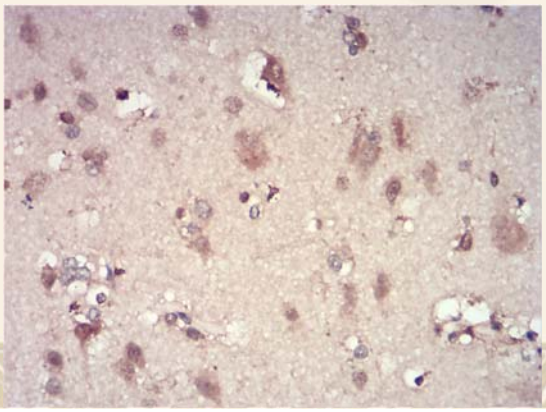
Adjacent normal kidney – M54, SAM



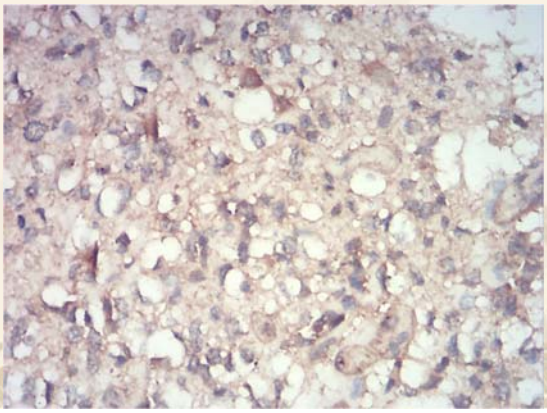
Kidney clear cell carcinoma (T1N0M0, I) – M54, SAM

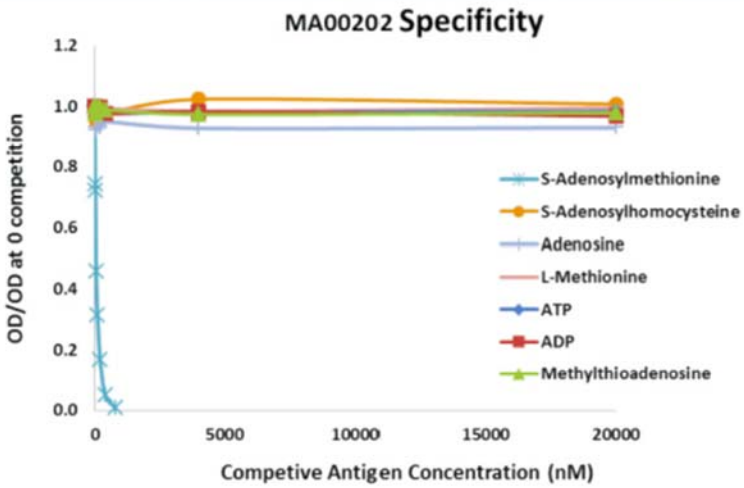


Adjacent normal cerebrum – M52, SAM



Astrocytoma – M18, SAM





Specificity of monoclonal anti-SAM antibody 84-3

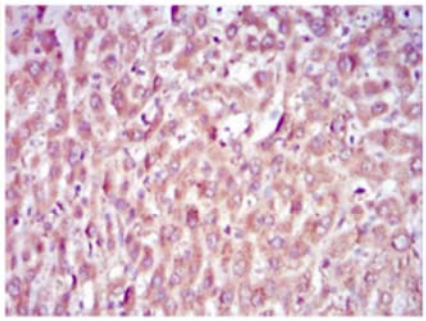


Figure 2 Immunohistochemistry staining performed using MA00202 with benign liver tissue adjacent to carcinoma. Brown areas indicated strong positive staining in cytoplasm (X400).

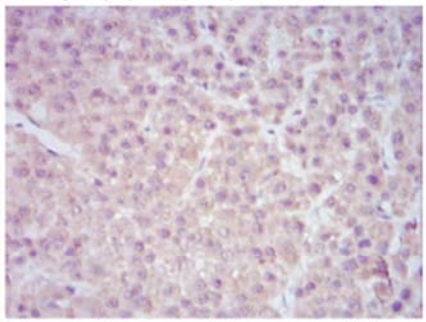
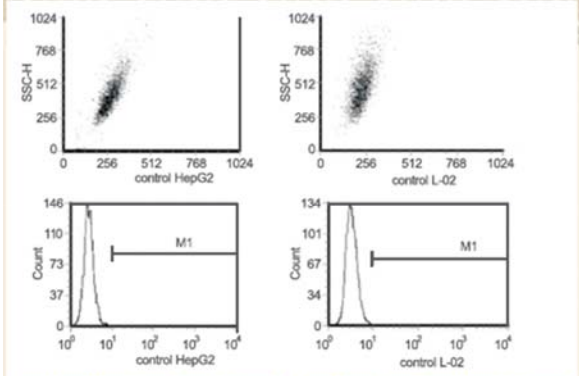


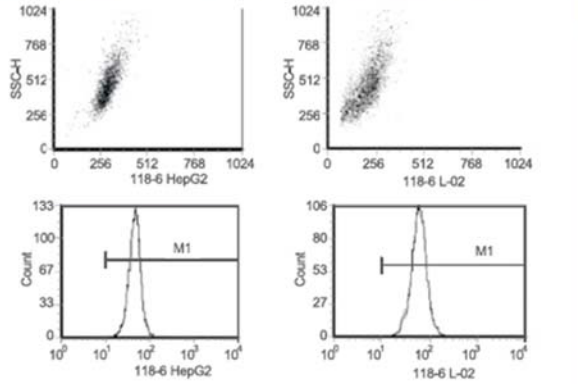
Figure 3 The immunohistochemical statining is performed for the same sample as in Figure 2 with liver cancer tissue. Cytoplasm showed background staining (further dilution beyond 1:200 is required) with Ma00202 (X400).



Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	2.72
M1	10	10000	17	0.17	48.98

Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	3.33
M1	10	10000	44	0.44	17.12

Figure 4 FCM analysis control. Normal liver cells L02 and carcinoma cells Hep G2 were stained with the buffer without any antibody.



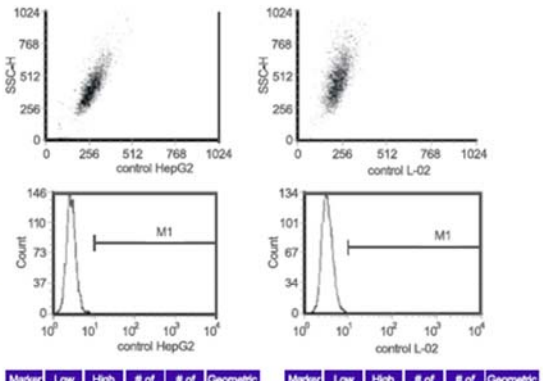
Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	45.34
M1	10	10000	9979	99.79	45.55

Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	58.22
M1	10	10000	9992	99.92	58.34

Figure 5 FCM results from normal liver cell line L02 and hepatocyte carcinoma cell line Hep G2 stained with anti-SAM monoclonal antibody from clone 118-6. Average fluorescence signal in Hep G2 cells was reduced compared to that in L02 cells, indicating SAM level is reduced during carcinogenesis.

Proof of concept: anti-SAM antibodies can be used in FCM. Not meant for quantification

Proof of concept: Anti-SAM antibody 84-3 stains better than 118-6 in FCM



Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
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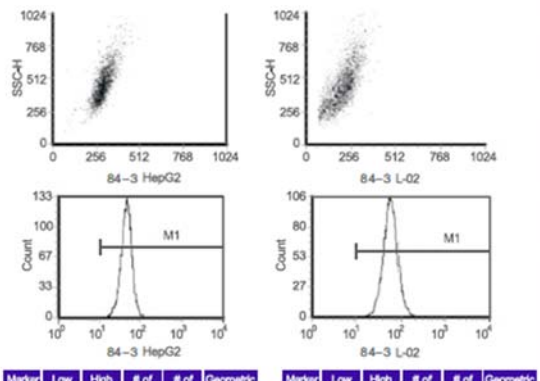
M1	10	10000	17	0.17	48.98

Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	3.33
M1	10	10000	44	0.44	17.12

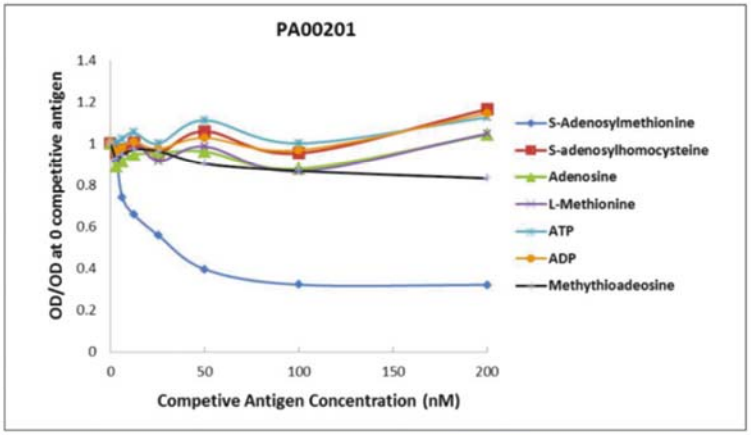
Figure 4 FCM analysis control. Normal liver cells L02 and carcinoma cells Hep G2 were stained with the buffer without any antibody.



Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	248.84
M1	10	10000	9993	99.93	249.65

Marker	Low bound	High bound	# of Events	# of gated cells	Geometric Mean
ALL	1	10000	10000	100.0	299.31
M1	10	10000	9992	99.92	300.38

Figure 5 FCM results from normal liver cell line L02 and hepatocyte carcinoma cell line Hep G2 stained with anti-SAM monoclonal antibody from clone 84-3. Average fluorescence signal in Hep G2 cells was reduced compared to that in L02 cells, indicating SAM level is reduced during carcinogenesis.



Polyclonal antibody against SAM

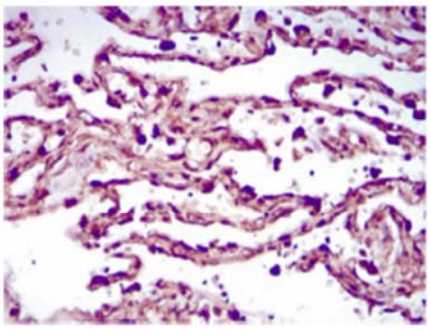


Figure 2 Immunohistochemistry staining performed using PA00201 (1:20) with benign lung tissue adjacent to carcinoma. Brown areas indicated strong positive staining in cytoplasm and nuclei (X400).

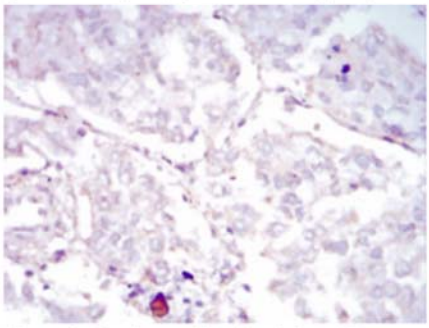


Figure 3 The immunohistochemical statining was performed for the same sample as in Figure 2 with lung cancer tissue. Cytoplasm and nucleus showed negative staining with PA00201 at 1:20 dilution (X400).

Functional Affinity – Mouse anti-SAM

For the reaction: Ab + Ag = AbAg
the rate of formation of the AbAg complex rf= Kforward[Ab][Ag]
the rate at which it breaks down rb= Kbackward[AbAg]
[Ag]: Concentration of antigen; [Ab]: concentration of antibody; [AgAb]: concentration of AgAb complex. The rate constants Kforward and Kbackward depends on temperature, pH and other conditions.

At equilibrium, rf = rb
The equilibrium or affinity constant Ka or K is defined from the equation:

$$K = \frac{k_{forward}}{k_{backward}} = \frac{[AbAg]}{[Ab][Ag]}$$

Dilution	Monoclonal anti-SAM 84-3 PLL-SAM	
	0.05ug/ml	0.1ug/ml
1000	3.3764	4.7244
2000	3.2469	4.5207
4000	3.1591	4.5844
8000	2.9176	4.3432
16000	2.6605	4.2673
32000	2.1978	3.7642
64000	1.6954	3.1152
128000	1.2151	2.3332

At 0.1ug/ml, half the maximum OD was seen at 1:130000. [Ab] = (1mg/ml/160,000g/mol)/130000 = 4.807×10^{-11}.
at 0.05ug/ml, half the maximum OD was seen at 1:7000. [Ab] t= (1mg/ml/160,000g/mol)/7000 = 8.92×10^{-11}.
n = (0.1ug/ml)/(0.05ug/ml) = 2

Ka = (n-1)/2*(n[Ab]-[Ab]t) = 7.29×10^{10} L/mol = 1.37×10^{-11} M

Sensitivity - Mouse anti-SAM

The data below showed the minimum detection limit, which was calculated by OD (when antigen = 0) – 2 x Standard deviation = 1.2683– 2 x 0.0.0439 = 1.1805, was about 2.6nM.

118-6

SAM (nM)	OD450	OD450	OD450	Mean	SD
Blank	0.0556	0.0808	0.0616	0.0660	0.0131
0	1.2189	1.2831	1.3030	1.2683	0.0439
6.25	1.110	1.0648	0.9757	1.0502	0.0683
12.5	0.885	0.8004	0.8737	0.8530	0.0459
25	0.6252	0.6435	0.6108	0.6265	0.0164
50	0.3838	0.3995	0.3782	0.3872	0.0110
Sensitivity	2.6 nM				

84-3

SAM standard (nM)	PLL-aza-SAM 0.2µg/ml			0.15µg/ml			0.1µg/ml		
Blank	0.0498	0.056	0.0522	0.0476	0.0471	0.0482	0.0754	0.0515	0.0476
0	1.4709	1.4344	1.4072	1.215	1.2484	1.2651	1.0844	1.1231	1.0633
6.25	1.365	1.3257	1.2673	1.0789	1.0734	1.0842	0.879	0.9122	0.8995
25	1.1769	1.1498	1.1015	0.8231	0.844	0.8334	0.6209	0.6561	0.6679
50	1.0230	0.9856	0.9577	0.6428	0.6362	0.6005	0.4517	0.4555	0.5187
100	0.7687	0.7532	0.7292	0.4292	0.4203	0.4427	0.3036	0.3103	0.3071
Sensitivity	3.0nM			1.7nM			1.6nM		

Mouse anti-SAH Cross Reactions to Analogs

S-Adenosylhomocysteine: 100%

S-Adenosylmethionine: 1.5 – 2.5%

Adenosine: L-Methionine: <1%

Adenosine Triphosphate: ~1.29%

Homocysteine: <1%

L-Cysteine: <1%

Glutathione: <1%

L-Cystathionine: <1%

Methythioadenosine (MTA): <5%

ADP (adenosine diphosphate): < 1%

ATP (adenosine triphosphate): < 1%

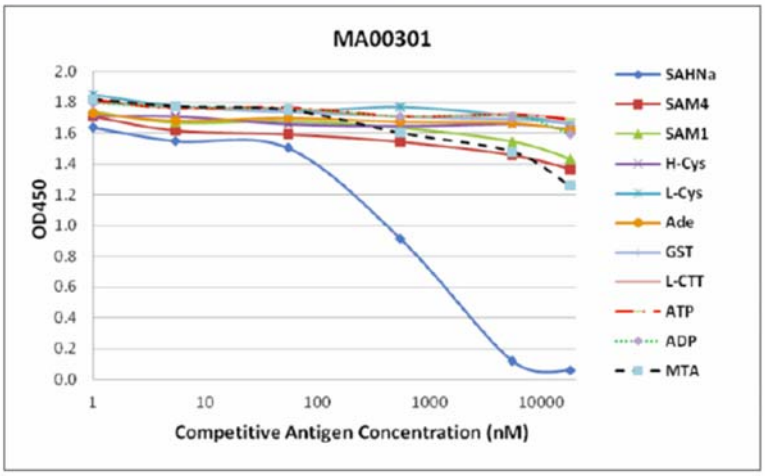


Figure 1 Competitive ELISA using anti-S-Adenosymethionine monoclonal antibody [301-1] (Ma00301)

Functional Affinity – Mouse anti-SAH (301-1)

Dilution from 2.31mg/ml	BSA-SAH 0.5µg/ml	BSA-SAH 0.25µg/ml
500	4.7390	4.3120
1000	4.9049	4.0379
2000	4.5869	3.7715
4000	4.1598	3.5240
8000	3.2419	2.7578
16000	2.3605	2.0027
32000	1.3452	1.2803
64000	0.9372	0.8287

At 0.5ug/ml, half the maximum OD was seen at 1:16000. [Ab] = (2.31mg/ml/160,000g/mol)/16000 = 9.023×10^{-10}

at 0.25ug/ml, half the maximum OD was seen at 1:12000. [Ab] t= (2.31mg/ml/160,000g/mol)/12000 = 1.203×10^{-9} M.

n = (0.5ug/ml)/(0.25ug/ml) = 2

$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 8.32 \times 10^{8}$ L/mol = 1.2×10^{-9}M

Functional Affinity – Mouse anti-SAH (844-1)

Dilution from 1mg/ml	BSA-SAH 0.2µg/ml	BSA-SAH 0.4µg/ml
1000	2.94505	3.79275
2000	2.71675	3.6411
4000	2.51445	3.5291
8000	2.34215	3.33185
16000	2.0829	3.07145
32000	1.74925	2.5685
64000	1.28195	1.8321
128000	0.9474	1.3221

At 0.4ug/ml, half the maximum OD was seen at 1:64620. [Ab] = (1mg/ml/160,000g/mol)/64620 = 9.67×10^{-11} M.
at 0.2ug/ml, half the maximum OD was seen at 1:51000. [Ab] t= (1mg/ml/160,000g/mol)/51000 = 1.225×10^{-10} M.
n = (0.5ug/ml)/(0.25ug/ml) = 2

$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 7.053 \times 10^9$ L/mol

Functional Affinity – Mouse anti-SAH (839-6)

Dilution from 1mg/ml	BSA-SAH 0.1µg/ml	BSA-SAH 0.2µg/ml
4000	1.9792	2.8577
8000	1.8630	2.7525
16000	1.7730	2.54815
32000	1.5913	2.2704
64000	1.3102	1.7912
128000	0.9864	1.2551
256000	0.6384	0.82805
512000	0.4205	0.5177

At 0.2ug/ml, half the maximum OD was seen at 1:110400. [Ab] = (1mg/ml/160,000g/mol)/110400 = 5.66×10^{-11} M.
at 0.1ug/ml, half the maximum OD was seen at 1:137500. [Ab] t= (1mg/ml/160,000g/mol)/137500 = 4.545×10^{-11} M.
n = (0.5ug/ml)/(0.25ug/ml) = 2

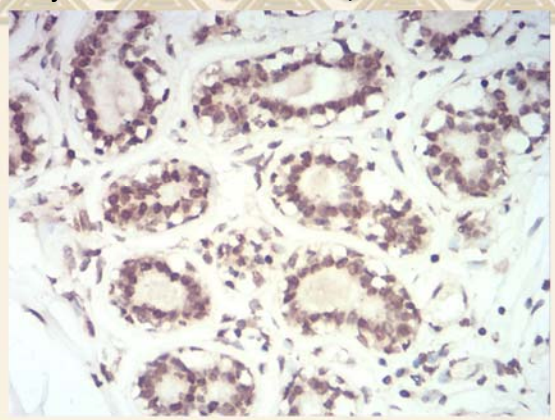
$K_a = (n-1)/2*(n[Ab] - [Ab]_t) = 7.38 \times 10^9$ L/mol

Sensitivity - Mouse anti-SAH

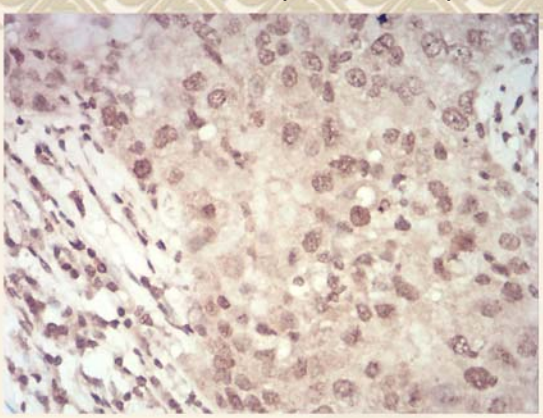
The data showed the minimum detection limit, which was calculated by OD (when SAH = 0) – 2 x Standard deviation = 0.8697– 2 x 0.033747 = 0.8022, was below 15.625nM.

SAH (nM)	OD450	OD450	OD450	Mean	Stdev	Inhibition (%)
250	0.4116	0.4426	0.442	0.4321	0.017727	50.32%
125	0.5421	0.6358	0.5372	0.5717	0.055566	34.26%
62.5	0.6055	0.7115	0.6444	0.6538	0.053622	24.82%
31.25	0.7285	0.8199	0.7545	0.7676	0.047094	11.74%
15.625	0.8045	0.8273	0.7975	0.8098	0.015582	6.88%
7.8125	0.7602	0.8113	0.7708	0.7808	0.026969	10.22%
3.90625	0.7722	0.9144	0.7786	0.8217	0.080315	55.52%
0	0.877	0.8992	0.8329	0.8697	0.033747	0%

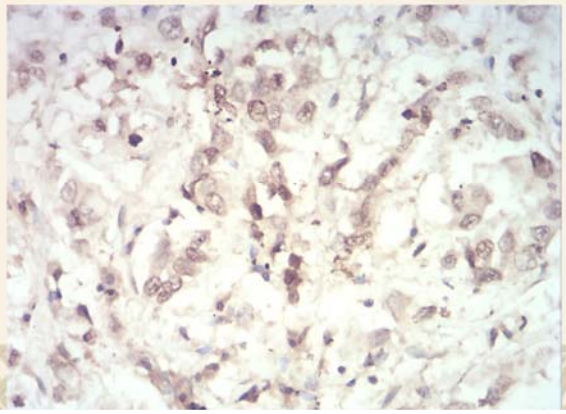
Adjacent normal breast – F46, SAH



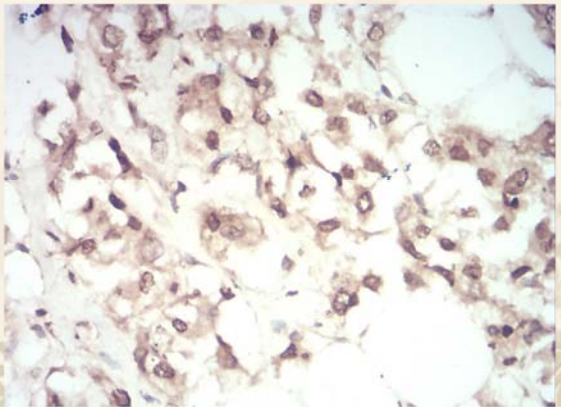
Invasive duct carcinoma (T2N1M0, IIb, G1) – F58, SAH



Invasive duct carcinoma (T4N1M0, IIIb, G2) – F50, SAH



Invasive duct carcinoma (T2N1M0, IIb, G3) – F56, SAH

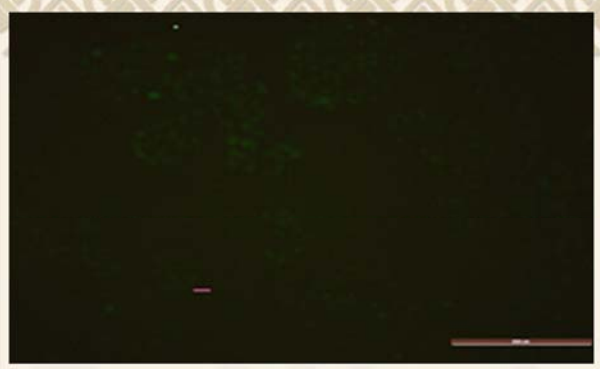


Part 3: Methionine Adenosyltransferase (MAT) Activity Assay

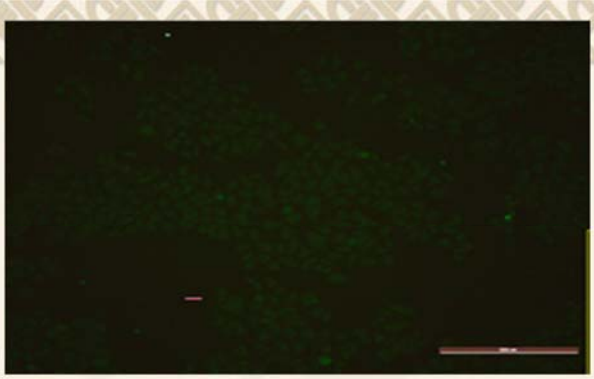
MAT Activity Assay kit is used to study MAT activity regulated by methionine (Met) and S-nitrosoglutathione (GSNO).

Except for parasites that rely on host for living, cells from all organisms have methionine adenyltransferase (MAT, EC2.5.1.6). MAT genes have been found to be exceptionally conserved throughout evolution. It was reported that there is 59% homology between human and *E. coli* MAT gene sequences. In mammals, three forms or isozymes of MAT have been identified that are encoded by three MAT genes. The MAT1a gene encodes α1 catalytic subunit. MAT-I is a tetramer of α1 subunits and MAT-III a dimer of the same subunits. Both MAT-I and MAT-III are present in adult liver cells. MAT-II is a heterotetramer formed by MAT2a encoding the catalytic subunit of α2 and MAT2b gene encoding regulatory β subunit, present in cells other than liver, embryonic liver and hepatoma cells.

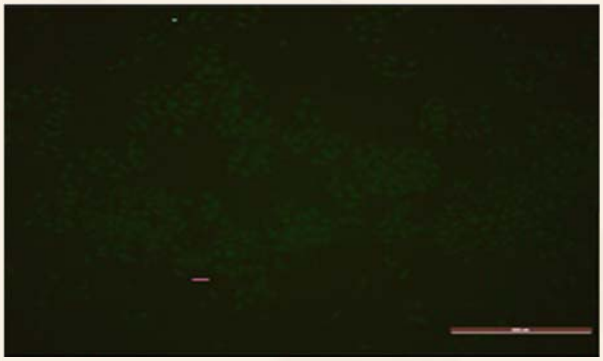
Regulation of MAT activity by methionine. L02 (expresses MAT1a) and HepG2 (expresses MAT2a and MAT2b) cells are good models to study the two types of MAT. **MAT-I/III is stimulated by Met to cope with high Met diet and inhibited by GSNO (S-nitrosoglutathione) which is a NO donor and critical signal in response to injury**. Like Reactive Oxygen Species (ROS), GSNO as a Reactive Nitrogen Species (RNS) is a free radical that imposes damages to cells, proteins and DNA. However, **MAT-II is inhibited by Met and has no response to GSNO.** All types of MAT catalyze SAM synthesis similarly.



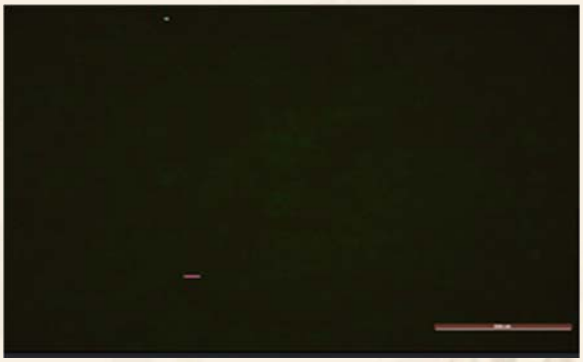
Anti-SAM antibody 1:400 L02: 0mM Met (x200)



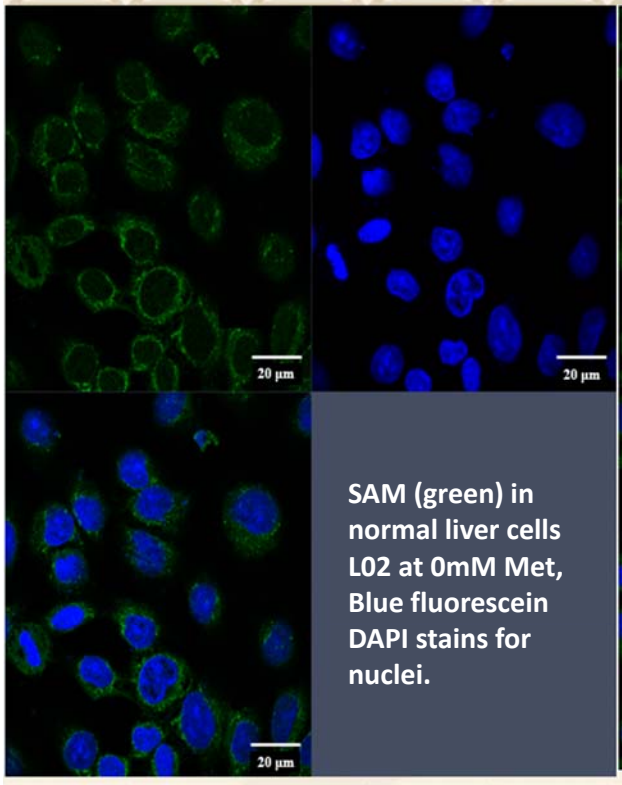
Anti-SAM antibody 1:400 L02: 0.5mM Met 24h (x200)



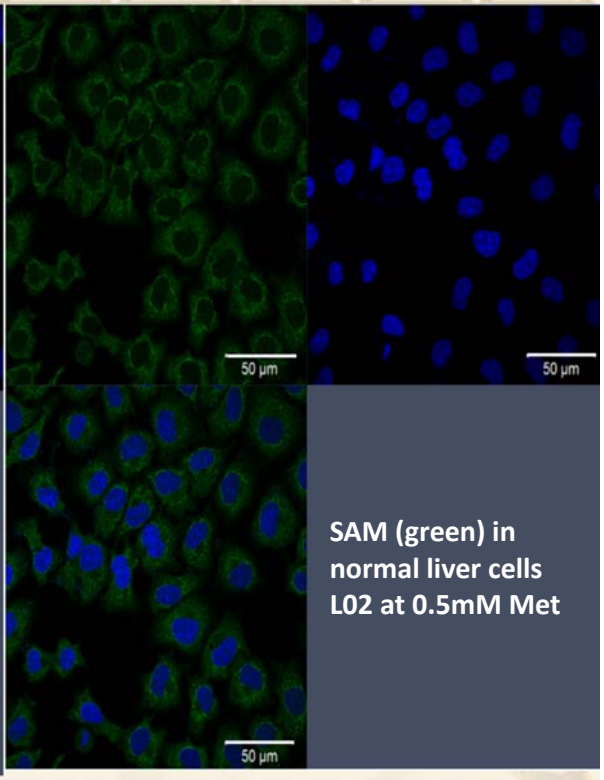
Anti-SAM antibody 1:400 HepG2: 0mM Met (x200)



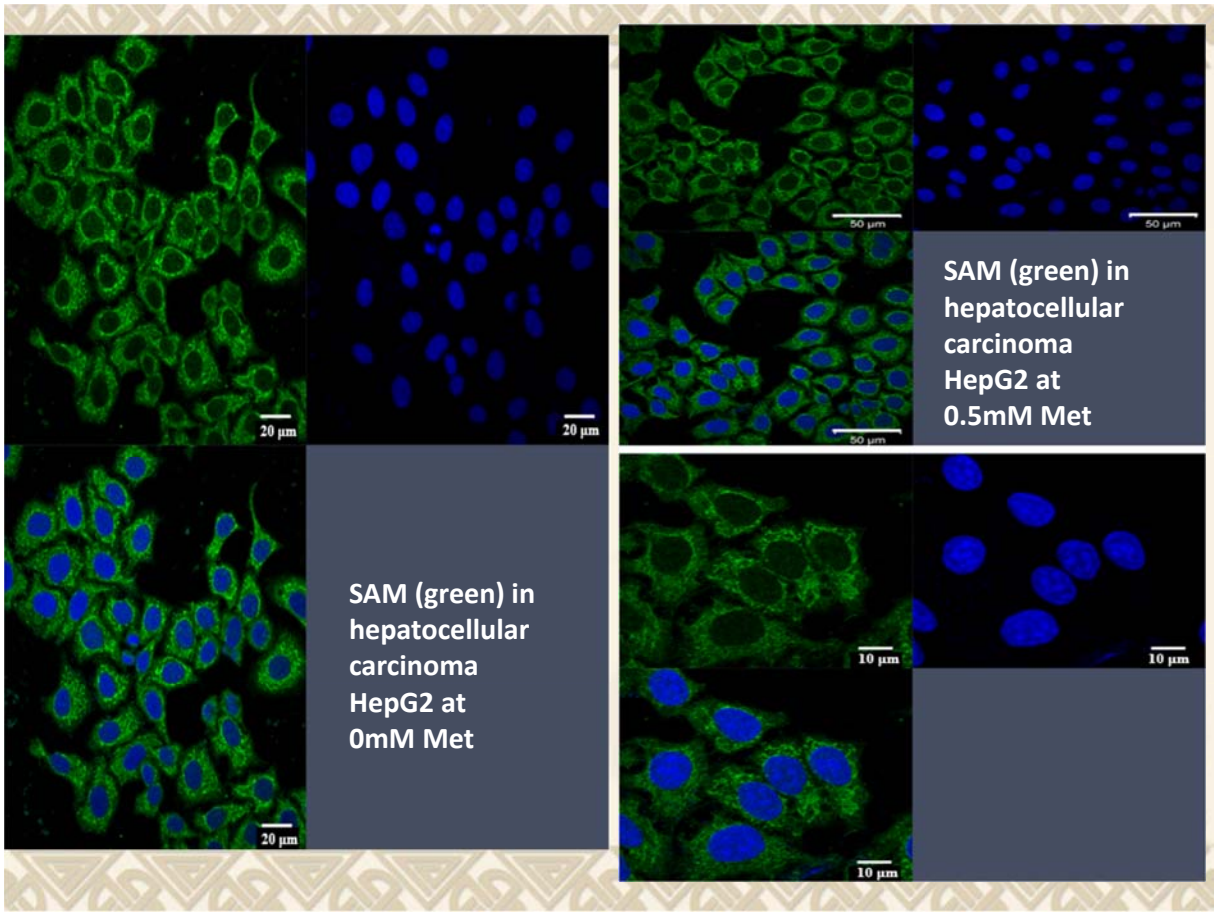
Anti-SAM antibody 1:400 HepG2: 0.5mM Met 24h (x200)

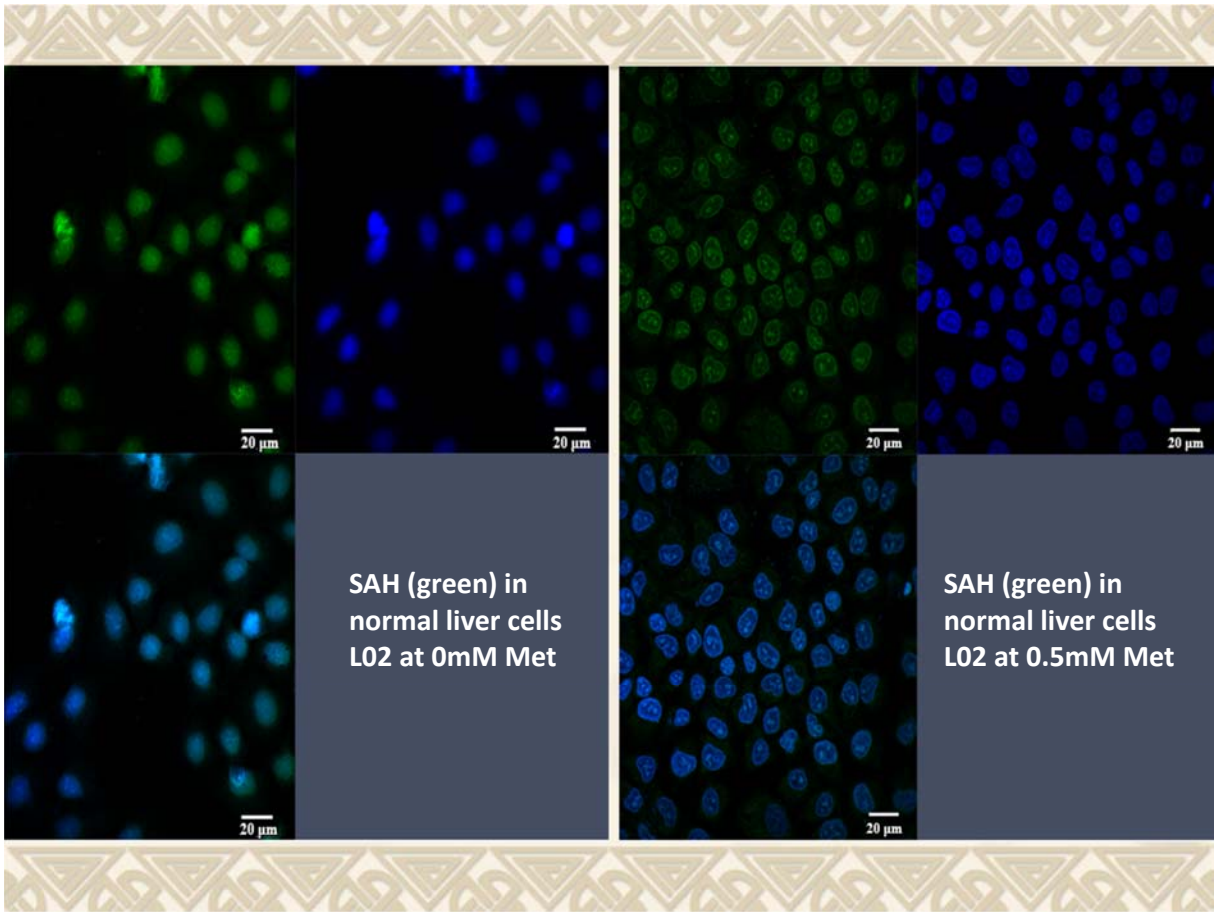


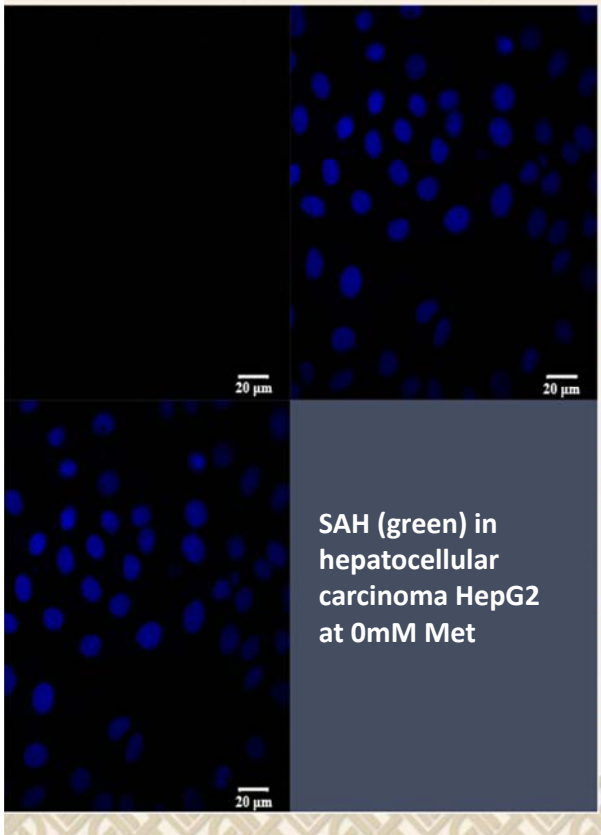
SAM (green) in normal liver cells L02 at 0mM Met, Blue fluorescein DAPI stains for nuclei.



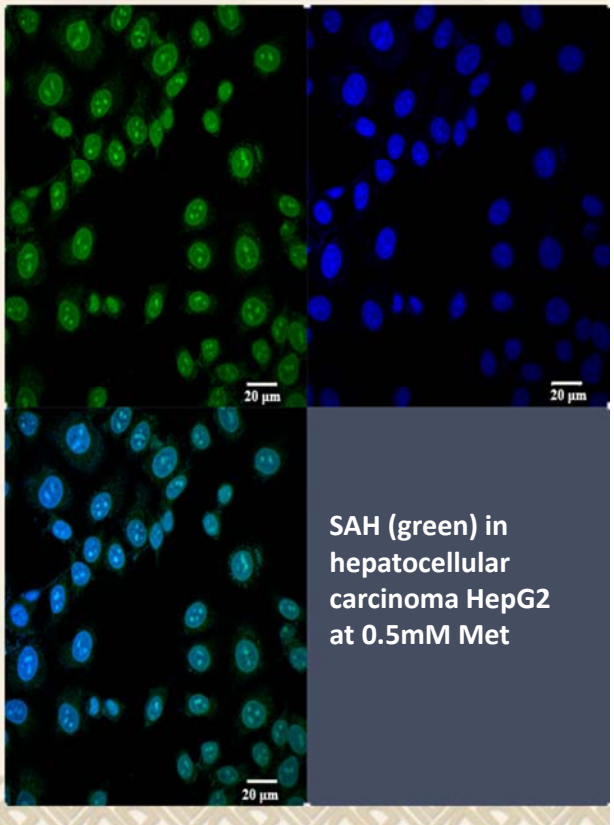
SAM (green) in normal liver cells L02 at 0.5mM Met


20 μm
20 μm
20 μm
SAM (green) in hepatocellular carcinoma HepG2 at 0mM Met
50 μm
50 μm
50 μm
SAM (green) in hepatocellular carcinoma HepG2 at 0.5mM Met
10 μm
10 μm
10 μm


20 μm
20 μm
20 μm
SAH (green) in normal liver cells L02 at 0mM Met
20 μm
20 μm
20 μm
SAH (green) in normal liver cells L02 at 0.5mM Met

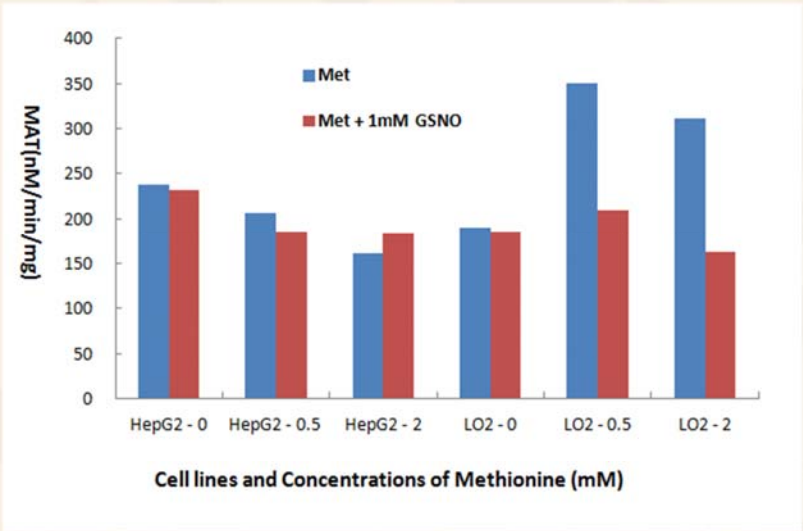


SAH (green) in hepatocellular carcinoma HepG2 at 0mM Met



SAH (green) in hepatocellular carcinoma HepG2 at 0.5mM Met

Regulation of MAT Activities by Met and GSNO in L02 and HepG2 Cells



Part 4: Products

Arthus
BIOSYSTEMS

Respond to challenge, make it possible

Streamline Biotech Research
New Tools for S-Adenosylmethionine and Methylation Index

Arthus Biosystems
Tel:+1-510-846-1804
Email:info@arthusbio.com
Web:www.arthusbio.com

Fast, easy and convenient way of measuring methylation index is possible

Stabilized SAM and SAH antigens and antigen conjugates

Aza-SAM a/b

Product name	Aza-SAM, Aza-SAM a/b
Catalog Number	AST00201, AST00201-1, AST00201-200
Chemical name	5'-N-Methyl, 5'-N-butyryl-5'-deoxyadenosine, or 5'-[(3-carboxypropyl)methylamino]-5'-deoxy-adenosine

PLL–aza–SAM a/b

Product name	PLL-aza-SAM a/b
Catalog Number	ACT00201-5/10
Description	Poly-lysine was conjugated to S-adenosylmethionine analog Aza-SAM

BSA–aza–SAM a/b

Product name	BSA-aza-SAM a/b
Catalog Number	ACT00204-5/10
Description	Bovine serum albumin (BSA) was conjugated to the S-adenosylmethionine analog Aza-SAM

SAH–Na

Product name	SAH-Na
Catalog Number	AST00301-1
Chemical name	S-Adenosylhomocysteine sodium salt
Description	SAH-Na was synthesized by adding sodium to the carboxyl group of S-adenosylhomocysteine.

BSA–SAH

Product name	BSA-SAH
Catalog Number	ACT00301-50
Description	Bovine serum albumin (BSA) was conjugated to S-adenosylhomocysteine (SAH).

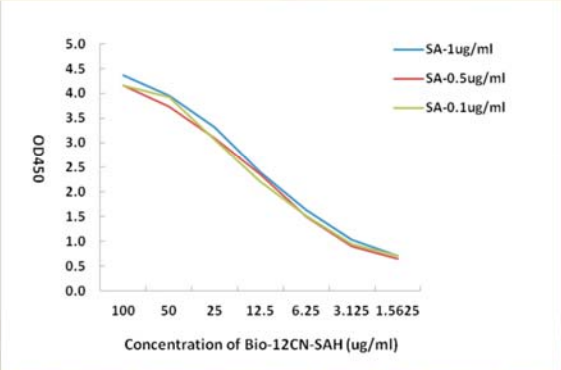
Biotin-conjugated SAH and Digoxin-conjugated SAH

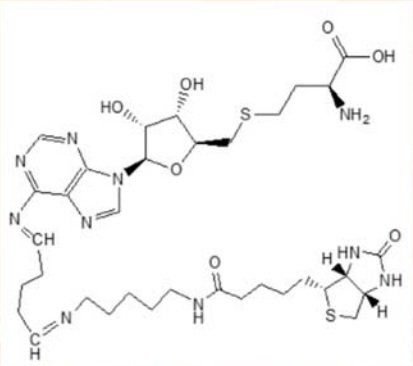
Dig–6C–SAH 1a/b/c

Product name	Dig-6C-SAH 1 a/b/c
Catalog Number	ACT00304-25/50/100
Description	Digoxin-conjugated S-adenosylhomocysteine: Digoxin is conjugated to NH2 of SAH through 6-bromocaproic acid.

Bio–12CN–SAH 1a/b/c

Product name	Bio-12CN-SAH 1a/b/c
Catalog Number	ACT00305-10/50/100
Description	Biotin is conjugated with S-adenosylhomocysteine through a 10-carbon and 2-nitrogen linker.





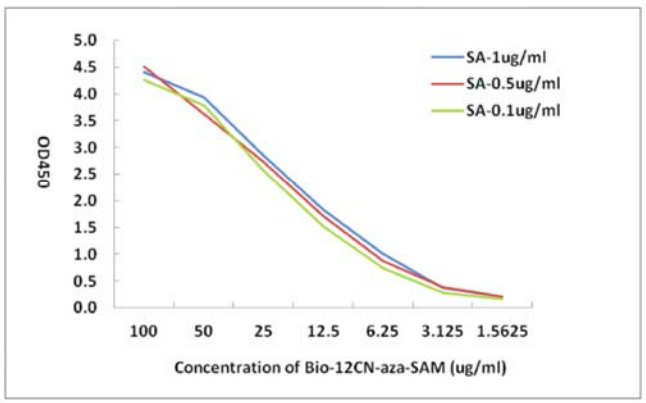
Biotin-conjugated SAM and aza-SAM

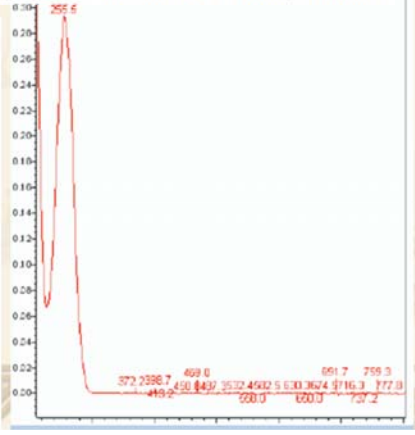
Bio–6C–SAM 1a/b

Product name	Bio-6C-SAM 1a/b
Catalog Number	ACT00203-50/100
Description	Biotin-conjugated S-adenosylmethionine:

Bio–12CN–aza–SAM 1a/b/c

Product name	Bio-12CN-aza-SAM 1a/b/c
Catalog Number	ACT00206-10/50/100
Description	Biotin is conjugated with aza-SAM through a 10-carbon and 2-nitrogen linker.





Arthus Biosystems, LLC 2600 Hilltop Dr. Building B Suite C239, Richmond, CA 94806.
USA: 844-486-3175. Other locations: support@arthusbio.com
Http://www.arthusbio.com

Arthus BIOSYSTEMS

Mouse anti-SAM 1a/b

Product name	**Mouse anti-SAM 1a/b**
Catalog Number	MA00201-50/100
Description	Mouse monoclonal antibody to S-Adenosylmethionine [118-6]
Specificity	Dosage-dependent competition was detected as a sample was added to a cELISA (Any SAM from a sample competes with the coated SAM heptan to bind HRP-conjugated antibody 118-6) . The sample is the product of the following biochemical reaction: Methionine Adenosyltransferase (MAT) was added to methionine and adenosine triphosphate under an appropriate buffer at 37°C. It indicates that antibody 118-6 specifically binds physiologically produced SAM.
Cross Reaction	MA00201 shows the following reactivity with related compounds: S-Adenosylmethionine: 100%, S-Adenosylhomocysteine: < 1%, Adenosine: < 1%, L-Methionine: < 1%, Methythioadenosine (MTA) : < 1%, ADP (adenosine diphosphate) < 1%, ATP (adenosine triphosphate) < 1%
Immunogen	S-Adenosylmethionine analog conjugated to KLH

Properties

Form	Liquid
Storage instructions	Store at 4°C, -20°C for long term storage
Storage buffer	PBS 10mM pH7.4 (NaCl 150mM), Sodium azide 0.02%, BSA 10mg/ml or PBS 10mM pH7.4 (NaCl 150mM), Sodium azide 0.02%, Glycerol 50%, BSA 10mg/ml
Purity	>95% Purified from mouse ascites fluid by affinity chromatography
Clonality	Monoclonal
Clone number	118-6
Immunoglobin isotype	IgG2b
Affinity	Ka = 5.75 x 10^{9}L/mol (1.74 x 10^{-10}M)
Research Areas	Methylation of biomolecules (DNA, RNA, proteins, hormones, neurotransmitters, etc.) One-carbon metabolism Signal Transduction

Arthus Biosystems, LLC 2600 Hilltop Dr. Building B Suite C239, Richmond, CA 94806.
USA: 844-486-3175. Other locations: support@arthusbio.com
Http://www.arthusbio.com

Arthus BIOSYSTEMS

Mouse anti-SAH 1a/b

Product name	**Mouse anti-SAH 1a/b**
Catalog Number	MA00301-50/100
Description	Mouse monoclonal antibody against S-Adenosylhomocysteine [301-1]
Specificity	MA00301 shows the following reactivities with related compounds: S-Adenosylhomocysteine: 100%, S-Adenosylmethionine: ~1.5%, Adenosine: <1 %, Homocysteine: < 1%, L-Cysteine: < 1%, Glutathione: < 1%, L-Cystathionine: < 1%, Methythioadenosine (MTA): < 5%, ADP (adenosine diphosphate): < 1%, ATP (adenosine triphosphate): < 1%.
Immunogen	S-Adenosylhomocysteine conjugated to BSA

Properties

Form	Liquid
Storage instructions	Store at 4°C, -20°C for long term storage
Storage buffer	PBS 10mM pH7.4 (NaCl 150mM), Sodium azide 0.02%, BSA 10mg/ml or PBS 10mM pH7.4 (NaCl 150mM), Sodium azide 0.02%, Glycerol 50%, BSA 10mg/ml
Purity	>95% Purified from mouse ascites fluid by affinity chromatography
Clonality	Monoclonal
Clone number	301-1
Immunoglobin isotype	IgG3
Affinity	Ka = 2.778 x 10^{8}L/mol (3.60 x 10^{-9}M)
Research Areas	Methylation of biomolecules (DNA, RNA, proteins, hormones, neurotransmitters, etc.) One-carbon metabolism Signal Transduction

S-Adenosyl-L-Methionine (SAM) ELISA Kit
(For Plasma, Serum or Tissue Samples)

Packaging size

96 tests

Detection range

30 nM – 960 nM

S-Adenosyl-L-Methionine (SAM) ELISA Kit

Packaging size

96 tests

Detection range

15 nM – 480 nM

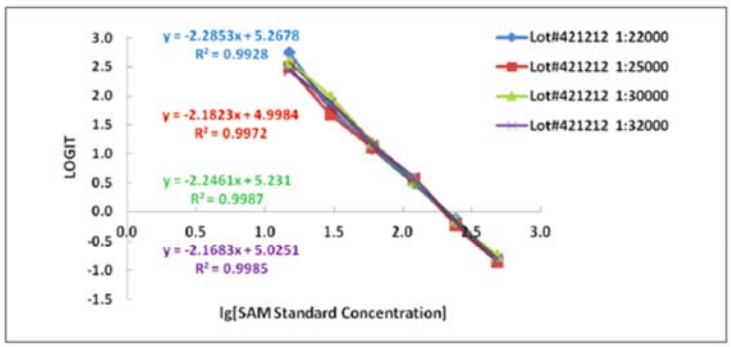


Figure 1 Standard curve Lot#421212 denotes the lot number of HRP-anti-SAM (clone 118-6) that was used at the different dilution shown above. LOGIT = $Ln(A/A_{S0}/(1-A/A_{S0}))$

S-Adenosyl-L-Homocysteine (SAH) ELISA Kit
(For Plasma, Serum or Tissue Samples)

Packaging size

96 tests

Detection range

15.625 nM – 1000 nM

S-Adenosyl-L-Homocysteine (SAH) ELISA Kit

Packaging size

96 tests

Detection range

15.625 nM – 1000 nM

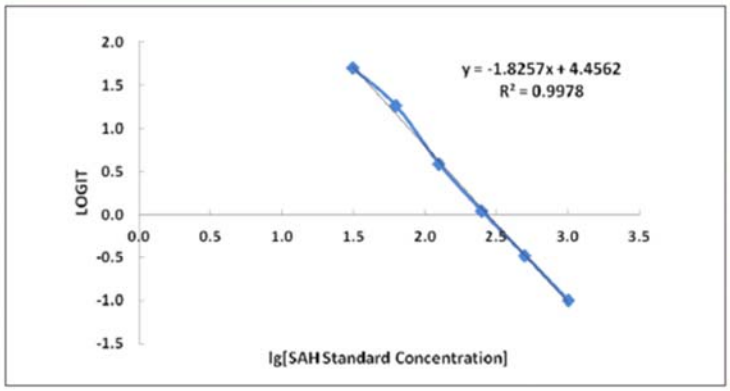
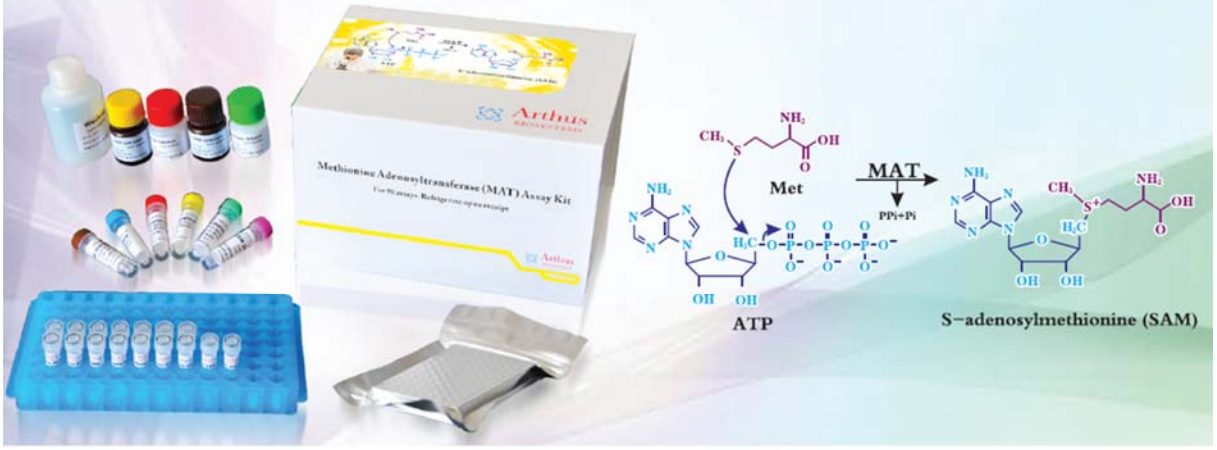


Figure 1 Standard curve in competitive ELISA with SAH standard from this kit
LOGIT = $Ln(A/A_{S0}/(1-A/A_{S0}))$

Methionine Adenosyltransferase (MAT) Activity Assay Kit



The first immunoassay kit to measure MAT activity accurately, conveniently and quickly

Enzymatic activity of MAT is critical in regulating the level of SAM and plays critical roles in methionine cycle and epigenetic study.

- Highly conserved MAT isoenzymes throughout evolution: MAT-I/III encoded by MAT1a gene and MAT-II encoded by MAT2a and MAT2b genes.
- MAT-II is important for cell proliferation
- SAM depleted animals show tissue injuries, necrosis and inflammatory infiltration
- Epigenetic regulation of MAT can be used as hepatocellular carcinoma therapy

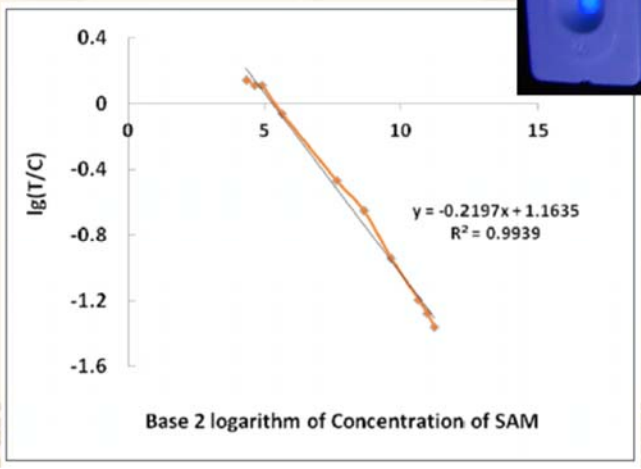
S-Adenosylmethionine (SAM) Quantitative Test Strip (Immunofluorescence Chromatography)

Product Name

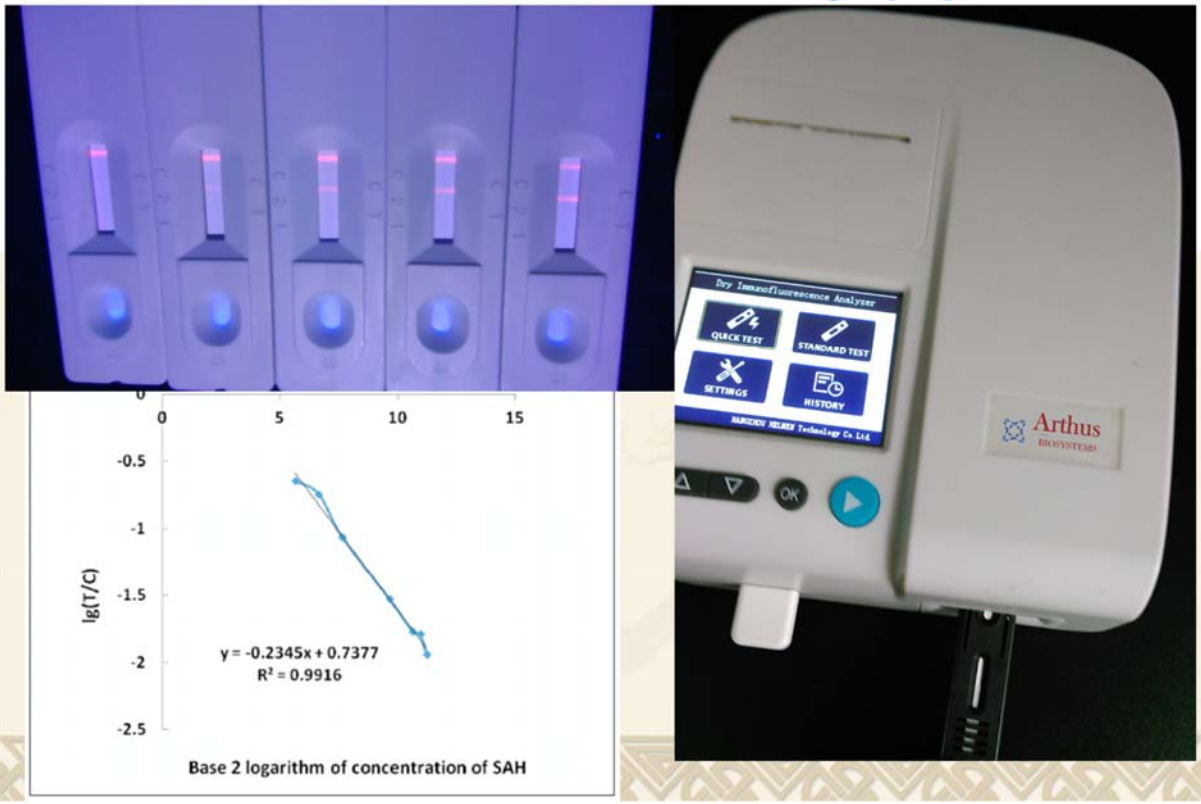
S-adenosylmethionine (SAM) Quantitative Test Strip (Im

Packaging

General packaging specifications: > 30 tests/pack.



S–Adenosylhomocysteine (SAH) Quantitative Test Strip (Immunofluorescence Chromatography)

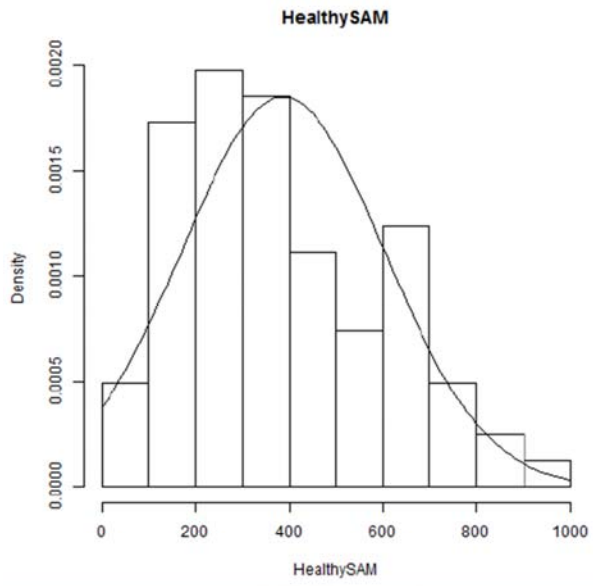


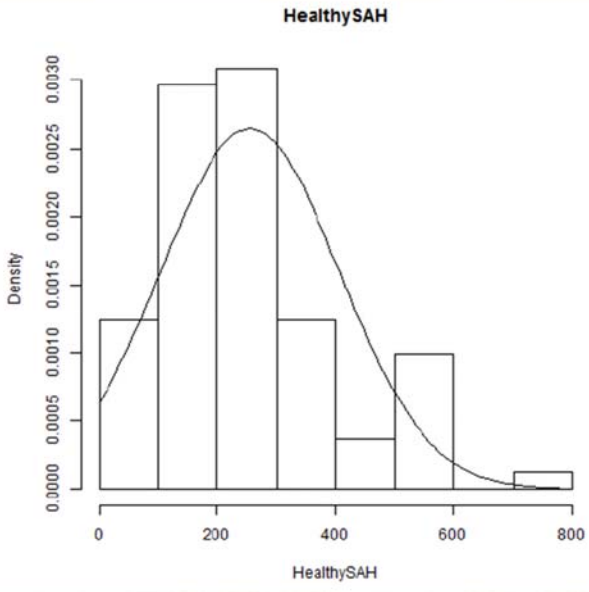
Part 5: Use SAM and SAH ELISA Kits in Human Plasma/Serum Samples

Importance of measuring SAM and Methylation Index (MI = SAM/SAH)

1. Biomarker and therapeutics for neurodegenerative diseases such as dementia, Parkinson's disease, amyotrophic lateral sclerosis (ALS), etc.
2. Serum SAM level for diagnosis of *Peumoncystis Carinii Pneumonia* (PCP) arising from immune compromised conditions.
3. Liver and bile duct diseases
4. Cancers
5. Nutritional imbalance such as folate and/or cobalamin deficiency
6. Congenital diseases such as Down syndrome and congenital heart diseases
7. Monitored treatment for depression, osteoarthritis and liver disorders with SAM-e

1. Serum Samples from 81 Normal Subjects and 99 Liver Diseases





Mean = 386.66 ± 216.20 nM

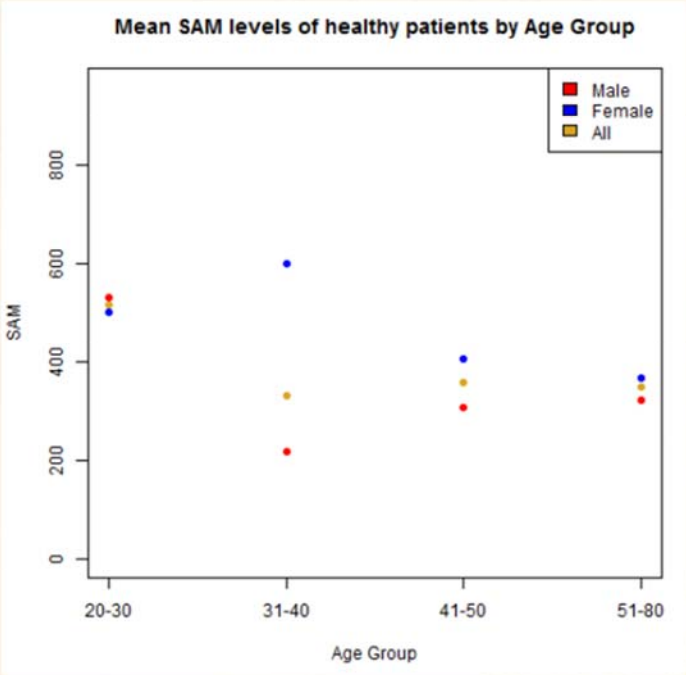
Mean = 217.30 ± 152.74 nM

MI = 3.75 ± 5.0 (0.1 – 9.9)

Normal SAM levels from 81 human sera are age and gender-dependent.

The younger the age, the higher the level of SAM. SAM level in age group younger than 30 is significantly higher than those from all other age groups. ($p<0.05$)

SAM level is generally higher in females than males.



ANOVA results

Dataset	Response Variable	Explanatory Variable	p-value	Significance
Healthy	SAM	Age Group	0.03437	* *
Healthy	SAH	Age Group	0.4964	
Healthy	MI	Age Group	0.1058	
Healthy	SAM	Gender	0.05115	*
Healthy	SAH	Gender	0.584	
Healthy	MI	Gender	0.8752	

Based on the ANOVA table, we see that Age Group is a significant factor with respect to SAM levels in healthy patients. So we will do pairwise t-tests for each pair of levels of Age Group.

Healthy sera

Age Group 1	Age Group 2	p-value	Significance
20-30	31-40	0.01728	* *
20-30	41-50	0.03793	* *
20-30	51-80	0.005682	* *
31-40	41-50	0.7461	
31-40	51-80	0.7815	
41-50	51-80	0.9081	

So clearly, by the pairwise t-test results, the SAM levels for the healthy patients aged 20-30 are statistically significant from those of the other age groups. And, looking at the plot, of mean SAM levels of healthy patients by Age Group, we see that the SAM levels of healthy patients aged 20-30 are significantly higher than those of the other age groups.

After doing a pairwise t-test for SAM levels of healthy patients by gender, we get a p-value of 0.05117 (note that this is approximately equal to the p-value we got from ANOVA) which is statistically significant at an $\alpha = 0.1$ level. The mean of the female SAM levels is 433.9628 and the mean of the male SAM levels is 340.5072 which means the SAM levels for healthy females are significantly higher than those of healthy males.

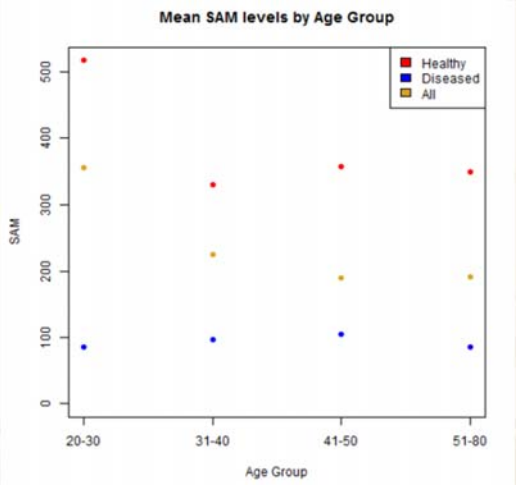
ANOVA Analysis of SAM, SAH and MI on 81 Healthy Subjects and 99 Liver Diseases

Dataset	Response Variable	Explanatory Variable	p-value	Significance
Healthy	SAM	Age Group	0.03437	* *
Healthy	SAH	Age Group	0.4964	
Healthy	MI	Age Group	0.1058	
Healthy	SAM	Gender	0.05115	*
Healthy	SAH	Gender	0.584	
Healthy	MI	Gender	0.8752	
Diseased	SAM	Age Group	0.9045	
Diseased	SAM	Gender	0.87	
Diseased	SAM	Diagnosis	0.2694	

SAM Levels in Diagnosis of Liver Diseases

Group	Case No.	SAM (nM)		SAM=120nM Cutoff		SAM=240nM Cutoff	
		Average	Standard Deviation	Detected Number	Detection Rate (%)	Detected Number	Detection Rate (%)
Normal	81	386.66	216.20	3	3.70	25	30.86
Hepatitis	46	101.42	83.12	37	80.43	44	95.65
Carcinoma	14	104.96	82.63	10	71.43	13	92.86
Cirrhosis	20	92.95	62.41	17	85.00	19	95.00
Liver failure	19	66.46	29.77	19	100	19	100

SAM level in liver diseases (99 cases) by age and gender.



Methylation Index and Cancers

Response Variable	Case #	Mean	p-value	Significance
SAM(LiverCancer)	23	278.0652	0.03451	**
SAH(LiverCancer)	23	293.35	0.3288	
MI(LiverCancer)	23	1.562109	0.1131	
SAM(LungCancer)	75	262.5039	0.001248	***
SAH(LungCancer)	75	326.9877	0.007807	***
MI(LungCancer)	75	0.9710702	3.674e-06	****
SAM(OtherCancer)	76	283.3233	0.00116	***
SAH(OtherCancer)	76	394.7301	5.612e-06	****
MI(OtherCancer)	76	0.8156412	8.123e-09	****

* represents significance at significance level = 0.1
** represents significance at significance level = 0.05
*** represents significance at significance level = 0.01
**** represents significance at significance level = 0.001

2. Lung Cancer : 80

t test (Lung cancer vs Normal)

Response Variable	Mean	p-value	Significance
SAM	137.6983	9.595e-16	****
SAH	462.522	1.028e-10	****
MI	0.3840688	1.079e-12	****

3. Methylation Index and Other Diseases >> (next)

Response Variable	Case #	Mean	p-value	Significance
SAM(Cerebral_hemorrhage)	48	357.9406	0.4365	
SAH(Cerebral_hemorrhage)		353.175	0.001237	***
MI(Cerebral_hemorrhage)		1.148035	2.308e-05	****
SAM(Diabetes)	43	262.3474	0.001136	***
SAH(Diabetes)		372.0686	0.003831	***
MI(Diabetes)		0.8619158	9.2e-08	****
SAM(HBP)	22	288.3645	0.0389	**
SAH(HBP)		358.1118	0.01378	**
MI(HBP)		0.9178064	3.929e-07	****
SAM(Heart_diseases)	51	315.962	0.07938	**
SAH(Heart_diseases)		440.9451	5.945e-07	****
MI(Heart_diseases)		0.7822701	5.609e-09	****
SAM(Inflammation)	35	223.732	3.052e-06	****
SAH(Inflammation)		292.012	0.2051	
MI(Inflammation)		0.8193137	1.905e-08	****
SAM(Kidney_diseases)	26	307.6073	0.1077	
SAH(Kidney_diseases)		497.1204	7.761e-06	****
MI(Kidney_diseases)		0.6976807	3.692e-09	****
SAM(Liver_diseases)	30	356.3407	0.5037	
SAH(Liver_diseases)		444.0027	1.22e-06	****
MI(Liver_diseases)		0.8921861	2.717e-07	****
SAM(Pulmonary_diseases)	36	393.3392	0.8827	
SAH(Pulmonary_diseases)		486.5144	4.24e-08	****
MI(Pulmonary_diseases)		0.7916811	5.271e-09	****

4. Methylation Index and Brain Disorders

Diseases	Case #	Response Variable	Mean	p-value	Significance
Cerebral hemorrhage	20	SAM	415.4277	0.6064	
Cerebral hemorrhage	20	SAH	363.3671	0.03015	**
Cerebral hemorrhage	20	MI	1.163449	2.027e-05	****
Depression	10	SAM	337.385	0.3531	
Depression	10	SAH	442.379	0.01176	**
Depression	10	MI	0.87051	4.806e-06	****
Parkinson's Disease	10	SAM	285.5726	0.07684	***
Parkinson's Disease	10	SAH	794.5792	0.06972	****
Parkinson's Disease	10	MI	0.74988	7.18e-06	****

5. Random Study - annual checkup of a group of people. Disease: 271 Healthy: 303

Diabetes + HBP Diabetes	19 (18 + 1)
High Blood Pressure (HBP)	41
Heart diseases	29
Inflammation + Gall Bladder diseases	16 (9 + 7)
Kidney diseases	15
Liver diseases + HBP Liver diseases	76 (75 + 1)
Others: Cerebral hemorrhage + Prostatic hypertrophy + Pulmonary diseases	22 (9 + 7 + 6)
Sub-healthy	53

Results of Student t-tests between healthy and each disease group

Response Variable	Mean	p-value	Significance
SAM (Healthy)	526.3652 (healthy 611.5152)	0.0001353	****
SAH (Healthy)	575.2576 (healthy 622.8114)	0.01636	**
MI (Healthy)	1.220361 (healthy 1.60444)	0.08857	*

Response Variable	Mean	p-value	Significance
SAM(Diabetes)	627.8685	0.7581	
SAH(Diabetes)	630.7851	0.8923	
MI(Diabetes)	2.212707	0.6306	
SAM(HBP)	435.4006	0.001295	***
SAH(HBP)	538.6896	0.03282	**
MI(HBP)	0.7906479	0.0001276	****
SAM(Heart_diseases)	394.0012	0.0002738	****
SAH(Heart_diseases)	583.9596	0.4597	
MI(Heart_diseases)	0.7189607	8.612e-05	****
SAM(Inflammation)	554.6392	0.3228	
SAH(Inflammation)	651.8661	0.61	
MI(Inflammation)	0.9227298	0.002366	***
SAM(Kidney_diseases)	507.8832	0.2275	
SAH(Kidney_diseases)	638.395	0.8118	
MI(Kidney_diseases)	0.7567159	0.0001006	****
SAM(Liver_diseases)	550.6897	0.05257	*
SAH(Liver_diseases)	624.0728	0.9643	
MI(Liver_diseases)	1.005925	0.004219	***
SAM(Others)	473.3247	0.05172	*
SAH(Others)	528.8568	0.09604	*
MI(Others)	1.056442	0.04746	**
SAM(Sub_healthy)	616.6034	0.8935	
SAH(Sub_healthy)	487.1439	0.000455	****
MI(Sub_healthy)	2.067991	0.2305	