Arthus® BIOSYSTEMS
Arthus Biosystems,LLC 13824 Leighfield St. Chantilly, VA 20151
USA Tel: +1 877-366-3888 Fax: +1 844-448-7410
E-mail: info@arthusbio.com Web: http://www.arthusbio.com

CERTIFICATE OF ANALYSIS

Product: Alexa Fluor® 488 (AF488) conjugated anti-S-adenosylhomocysteine (SAH) antibody clone 839-6

Catalog Number: MAF00302

Lot Number: Lot# MP480316

Document Release Date: March 16, 2016

Materials:

1. Alexa Fluor® 488: Life Technologies Cat# A20100
2. Chromatography buffer
3. Dialysis buffer
4. Column: Bio-Rad (1.0x 40cm)
5. Sephadex G-25 chromatography media: GE Healthcare
6. Peristaltic pump: Pharmacia P-1 type
7. UV detector: Beijing BINTA Instrument Technology Co.,Ltd., 8823B
8. Dialysis bag: molecular weight cutoff 12-14KDa
9. Magnetic stirrer: Fisher Scientific
10. Analytical Balance: OHAUS company, E10640
11. UV-visible spectrophotometer: Shanghai Jing-hua Technology Instrument Co., Ltd. 752
12. Adjustable thermostat rotating hybridization oven: SHEL LAB 1004 type
13. pH meter: Fisher Scientific accumet portable AP5
14. Swirl Mixer: VWR Mini vortexer MV1
15. Desktop cryogenic centrifuge: Eppendorf Company 5475 C type

Results:

The final product: Alexa Fluor® 488 conjugated mouse anti-SAH antibody 839-6
Concentration: 3.26mg /ml
Storage Buffer: 50mM Tris, 150mM NaCl, pH8.0, 0.5%BSA, 0.09% NaN3
Storage note: 2-8˚C from light to prevent fluorescence quenching.

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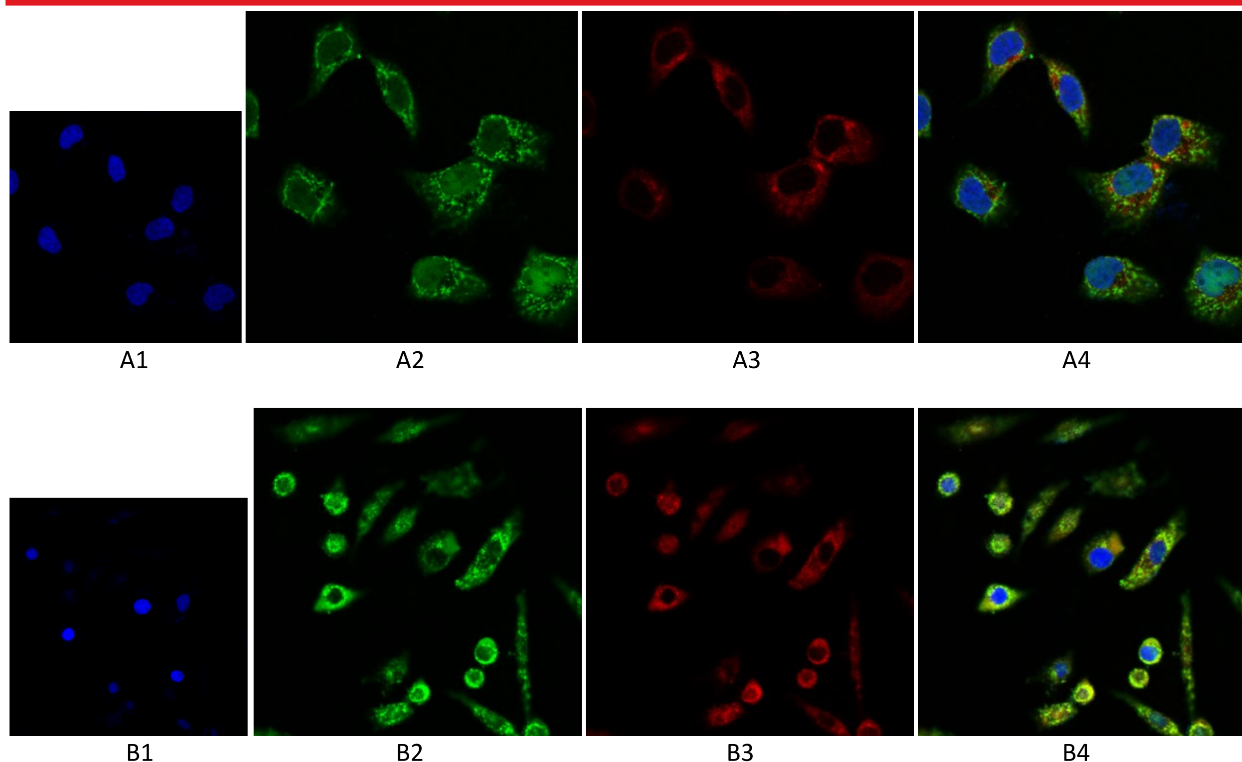

Figure 1 Immunofluorescence (IF) LSCM results of normal liver cells L02 cultured in RPMI 1640 with 10% FBS for 16h followed by stimulation by 0.5mM methionine for 24h (A1-A4) double stained with AF488-anti-SAH 839-6 (Cat# MAF00302) at 40µg/ml and R-PE-anti-SAM 84-3 (Cat# MAF00202) at 40µg/ml followed by DAPI staining. Hepatocellular carcinoma cell line HepG2 cells were cultured in RPMI 1640 with 10% FBS for 16h followed by stimulation by 0.5mM methionine for 24h (B1-B4) and double stained with AF488-anti-SAH 839-6 (Cat# MAF00302) at 60µg/ml and R-PE-anti-SAM 84-3 (Cat# MAF00202) at 60µg/ml followed by DAPI staining. Photography was performed under the laser scanning confocal microscope Zeiss LSM 780 (x630). Different views are as follows: DAPI (A1, B1); AF488 for SAH (A2, B2); R-PE for SAM (A3, B3); Overlap of all the three fluorescent signals (A4, B4). Expression patterns of SAM and SAH are different between L02 and HepG2 cells after methionine stimulation for 24h. In the case of cells not actively proliferating, both SAM and SAH are seen more in cytoplasm (more in mitochondria areas) than nuclear.

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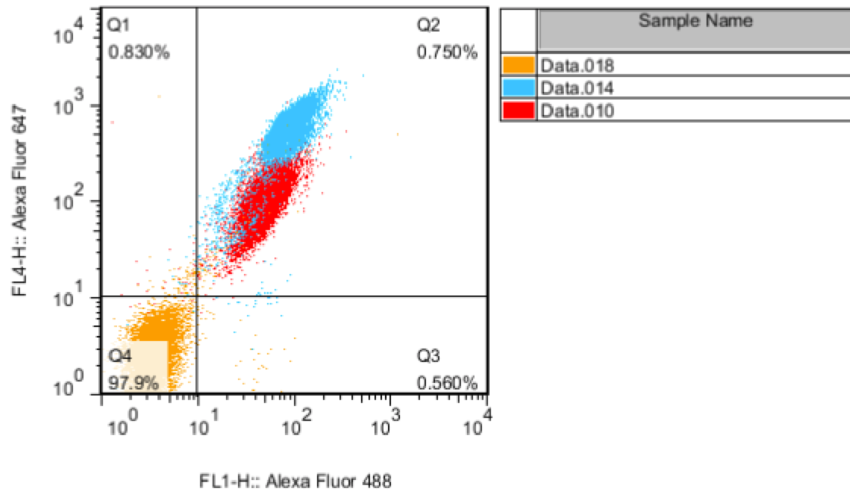
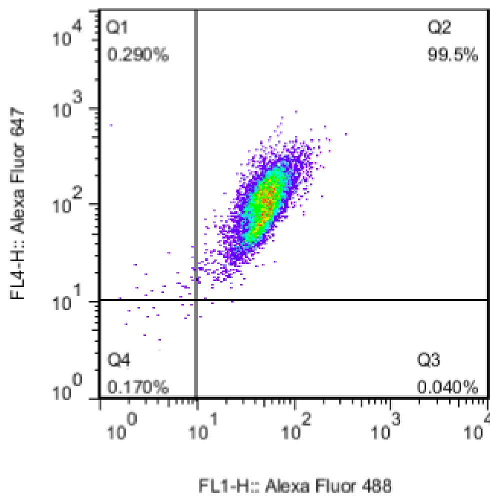
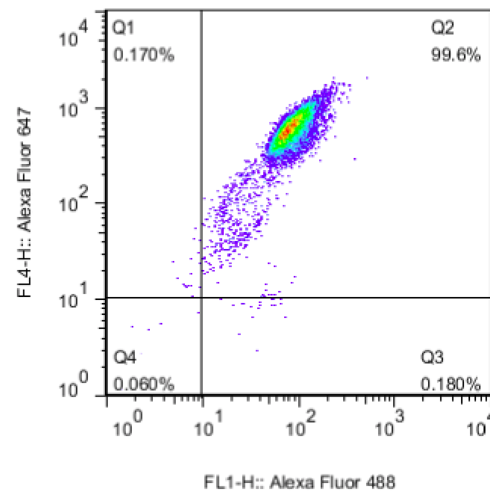


A1



A2



A3

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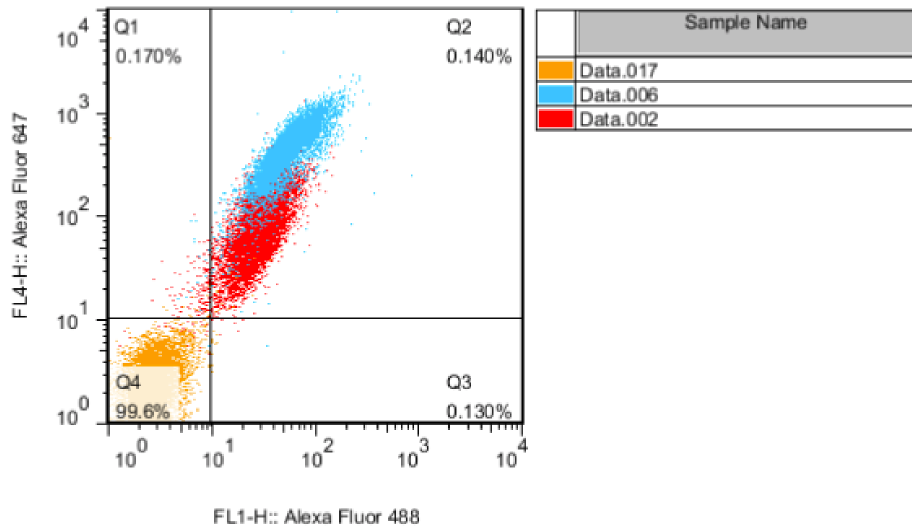
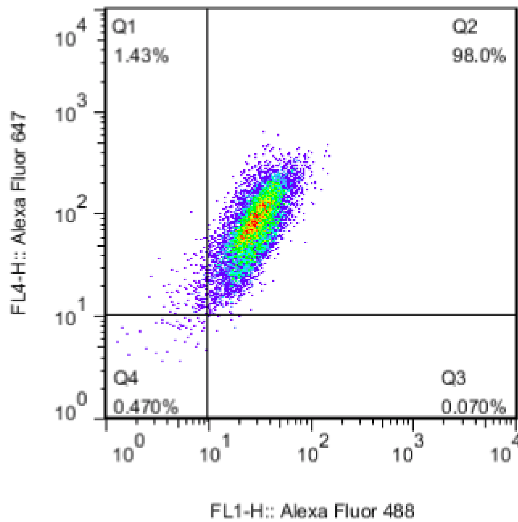
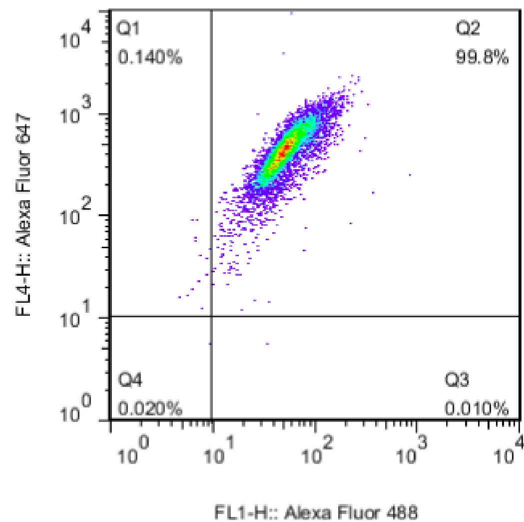


B1





B2

B3

Figure 2 Flow Cytometry of L02 (A1-A3) and HepG2 (B1-B3) cells double stained with Alexa Fluor® 488 conjugated anti-SAH antibody 839-6 (Cat# MAF00302) at 18 µg/ml and Alexa Fluor® 647 conjugated anti-SAM antibody 118-6 (Cat# MAF00201) at 4.5 µg/ml. Color legend: Orange: blank; Blue: nuclear

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fixation/permeabilization buffer was used (eBioscience 00-5523 FoxP3_TF Staining Buffer Set); Red: intracellular fixation/permeabilization buffer was used (eBioscience 00-8824). 100% confluent cells (cultured for 48h) were fixed and permeabilized with the intracellular fixation/permeabilization buffer (A2, B2) or the nuclear fixation/permeabilization buffer (A3, B3) and then double stained with antibodies indicated above. Cells were used for analysis with BD FACSCalibur Flow Cytometer. SAM expression is higher in L02 than HepG2 cells. Both SAM and SAH are expressed ubiquitously yet rather dynamically. The level of SAM is higher than that of SAH in both cells.

Hemans Chou
Quality Control Team

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