Arthus Biosystems, LLC 13824 Leighfield St. Chantilly, VA 20151
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CERTIFICATE OF ANALYSIS

Product: Alexa Fluor® 488 (AF488) conjugated anti-S-adenosylhomocysteine (SAH) antibody clone 301-3

Catalog Number: MAF00301

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 clone 301-3
Concentration: 3.2mg /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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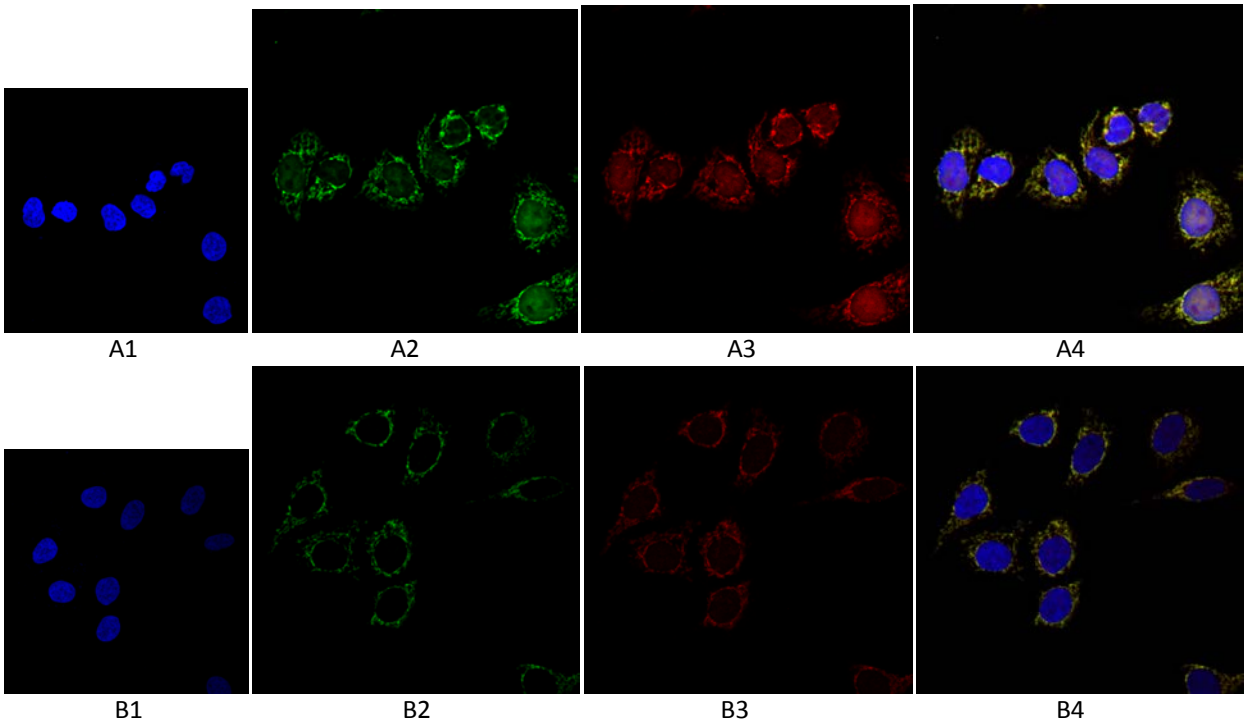

Figure 1 Immunofluorescence (IF) staining of HepG2 and L02 cells double stained with AF488-anti-SAH 301-3 (Cat# MAF00301) at 20µg/ml and AF647-anti-SAM 118-6 (Cat# MAF00201) at 4µg/ml followed by DAPI staining and photographed under the laser scanning confocal microscope Zeiss LSM 78 (x630). (A1-A4) Normal liver cell line L02 cells cultured in RPMI 1640 with 10% FBS for 40h. Different views are as follows: DAPI (A1); AF488 for SAH (A2); AF647 for SAM (A3); Overlap of all the three fluorescent signals (A4). (B1-B4) Hepatocellular carcinoma cell line HepG2 cells cultured for 40h (100% confluent). Different views are follows: DAPI (B1); AF488 for SAH (B2); AF647 for SAM (B3); Overlap of all the three fluorescent signals (B4). Both antibodies were used at relatively low concentration. When cells are 100% confluent, both SAM and SAH are mainly seen around nuclear membrane.

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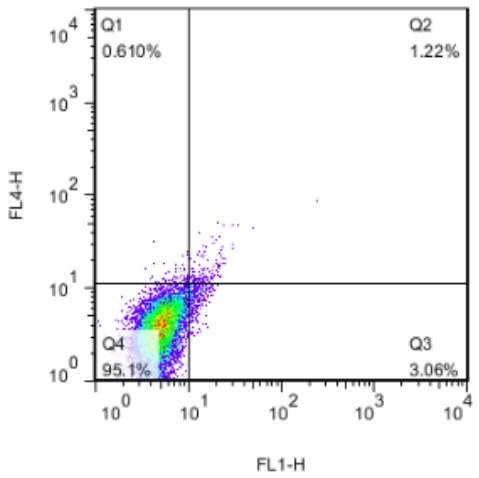
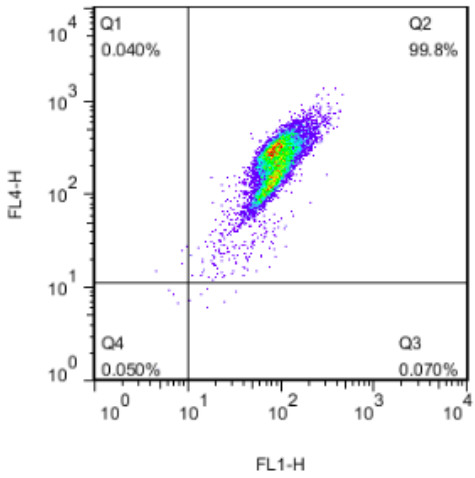


A1



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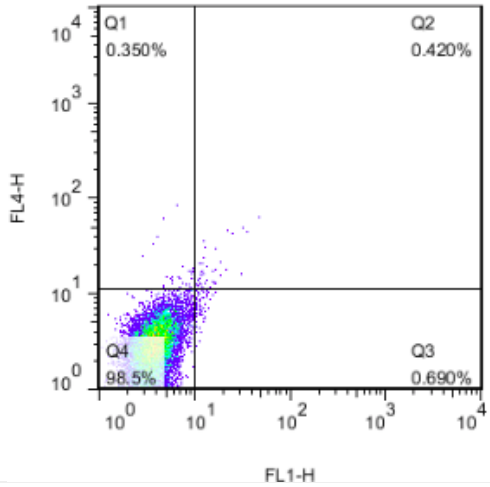
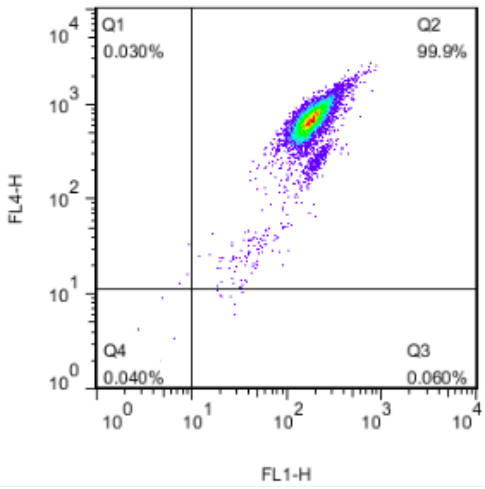


A3



A4

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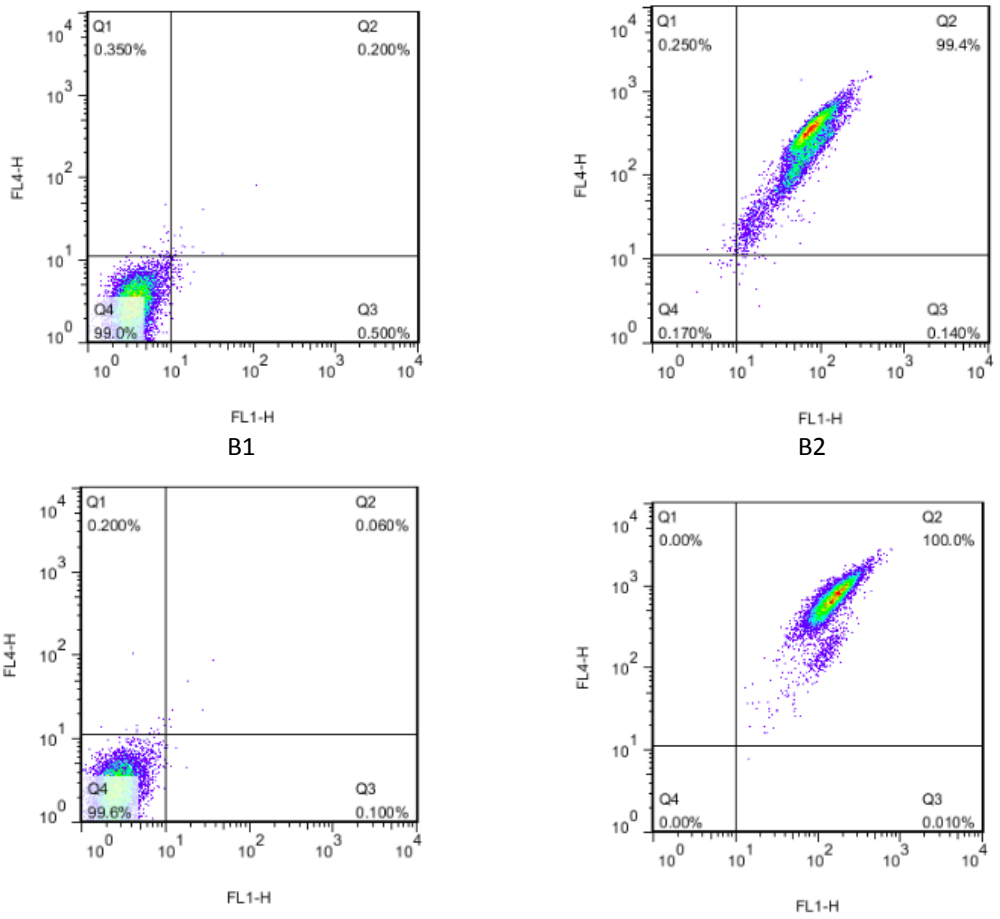

Figure 2 Flow Cytometry of L02 (A1-A4) and HepG2 (B1-B4) cells double stained with Alexa Fluor® 488 conjugated anti-SAH antibody 301-3 (Cat# MAF00301) at 28 µg/ml and Alexa Fluor®647 conjugated anti-SAM antibody 118-6 (Cat# MAF00201) at 4.5 µg/ml. 100% confluent cells (cultured for 48h) were fixed and permeabilized with the intracellular fixation/permeabilization buffer (A2, B2) or the nuclear fixation/permeabilization buffer (A4, B4) and then double stained with antibodies indicated above. A1 and B1 are the unstained control for A2 and B2 respectively. A3 and B3 are the unstained control for A4 and B4 respectively. Cells were used for analysis with BD FACSCalibur Flow Cytometer. Both fluorescent signals in A4 and B4 are higher than those of A2 and B2.

Hemans Chou
Quality Control Team