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CERTIFICATE OF ANALYSIS

Product: Alexa Fluor®647 (AF647) conjugated anti-S-adenosylmethionine (SAM) antibody clone 118-6

Catalog Number: MAF00201

Lot Number: Lot# MP480316

Document Release Date: March 16, 2016

Materials:

1. Alexa Fluor® 647: Life Technologies Cat# A20106
2. Chromatography buffer
3. Dialysis buffer
4. Column: Bio-Rad (1.0x 40cm)
5. Sephacryl S-200 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®647 conjugated mouse anti-SAM antibody clone 118-6
Concentration: 2.0mg /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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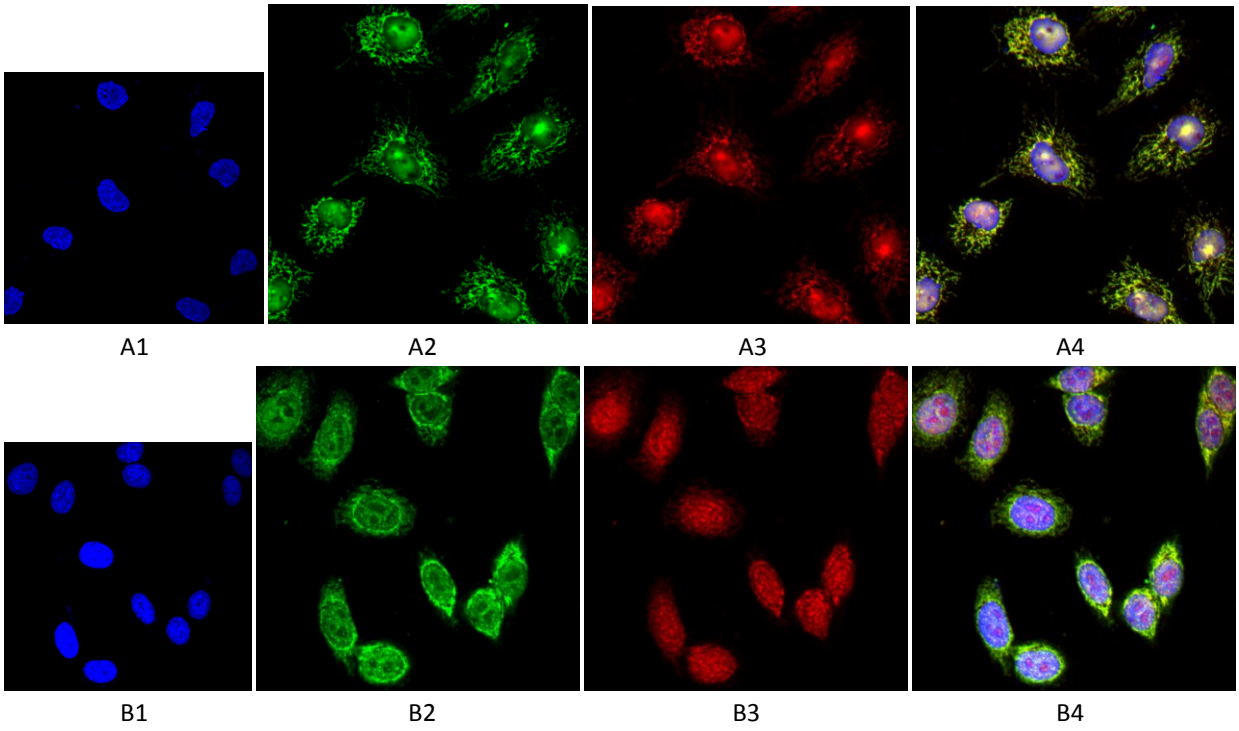
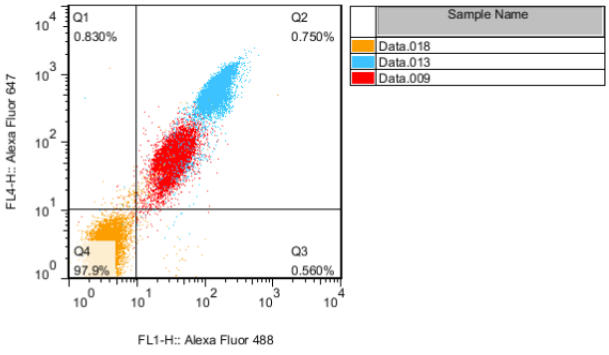


Figure 1 Immunofluorescence (IF) staining of HepG2 and L02 cells double stained with AF488-anti-SAH 301-3 (Cat# MAF00301) at 40µg/ml and AF647-anti-SAM 118-6 (Cat# MAF00201) at 8µg/ml followed by DAPI staining and photographed under the laser scanning confocal microscope (Zeiss LSM 780). (A1-A4) Normal liver cells L02 cells cultured 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. Different views are follows: DAPI (B1); AF488 for SAH (B2); AF647 for SAM (B3); Overlap of all the three fluorescent signals (B4). Expression patterns of SAM and SAH are different between L02 and HepG2 cells (x630).



A

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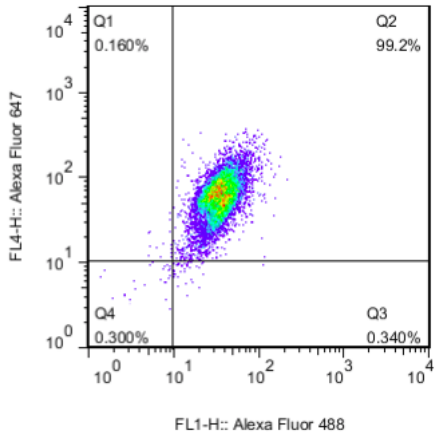

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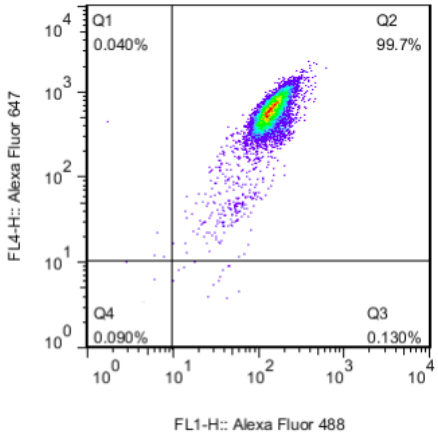
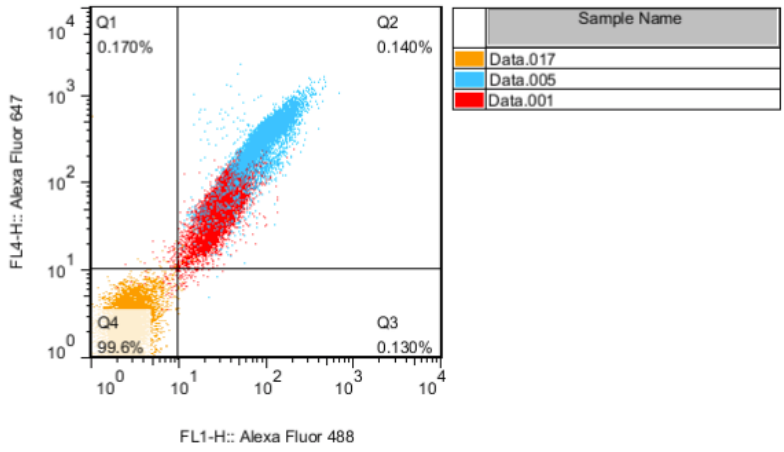


A3



B1

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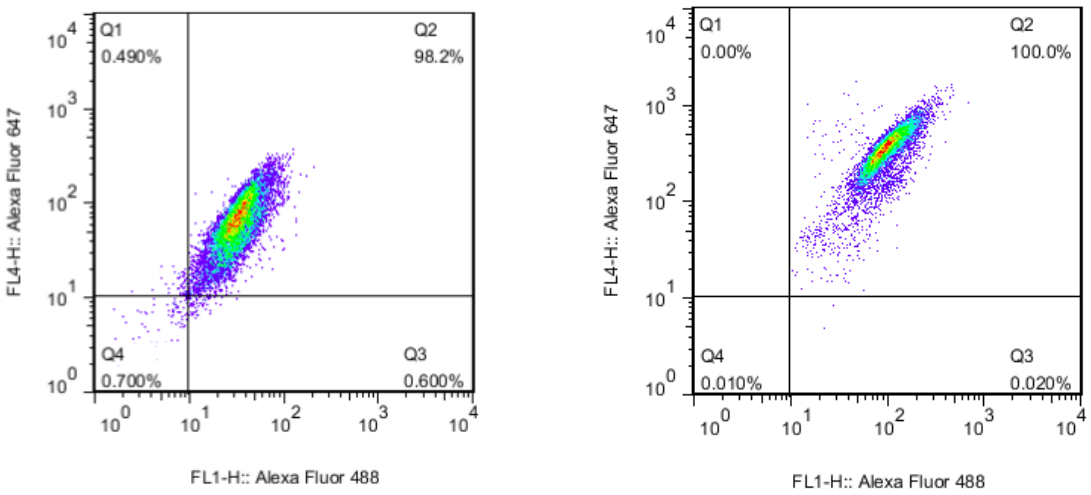

B2 B3

Figure 2 Flow Cytometry of L02 (A1-A3) and HepG2 (B1-B3) cells double stained with Alexa Fluor® 488 conjugated anti-SAH antibody 301-3 (Cat# MAF00301) at 36 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 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 in RPMI 1640 with 10% FBS 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