
CERTIFICATE OF ANALYSIS

Product: R-Phycoerythrin (R-PE) conjugated anti-S-adenosylmethionine (SAM) antibody clone 84-3

Catalog Number: MAF00202

Lot Number: Lot# MP480316

Document Release Date: March 16, 2016

Materials:

1. R-Phycoerythrin: Prozyme Cat# PB31
2. Chromatography buffer
3. Dialysis buffer
4. Column: Bio-Rad (1.0x 40cm)
5. Sephadex G-25 chromatography media: GE Healthcare;
Sephacryl S-300 chromatography media: Bio Rad;
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: R-Phycoerythrin conjugated mouse anti-SAM antibody clone 84-3

Concentration: 2.4mg /ml

Storage Buffer: 50mM Tris, 150mM NaCl, pH8.0, 0.5%BSA, 0.09% Na₃

Storage note: 2-8°C from light to prevent fluorescence quenching.

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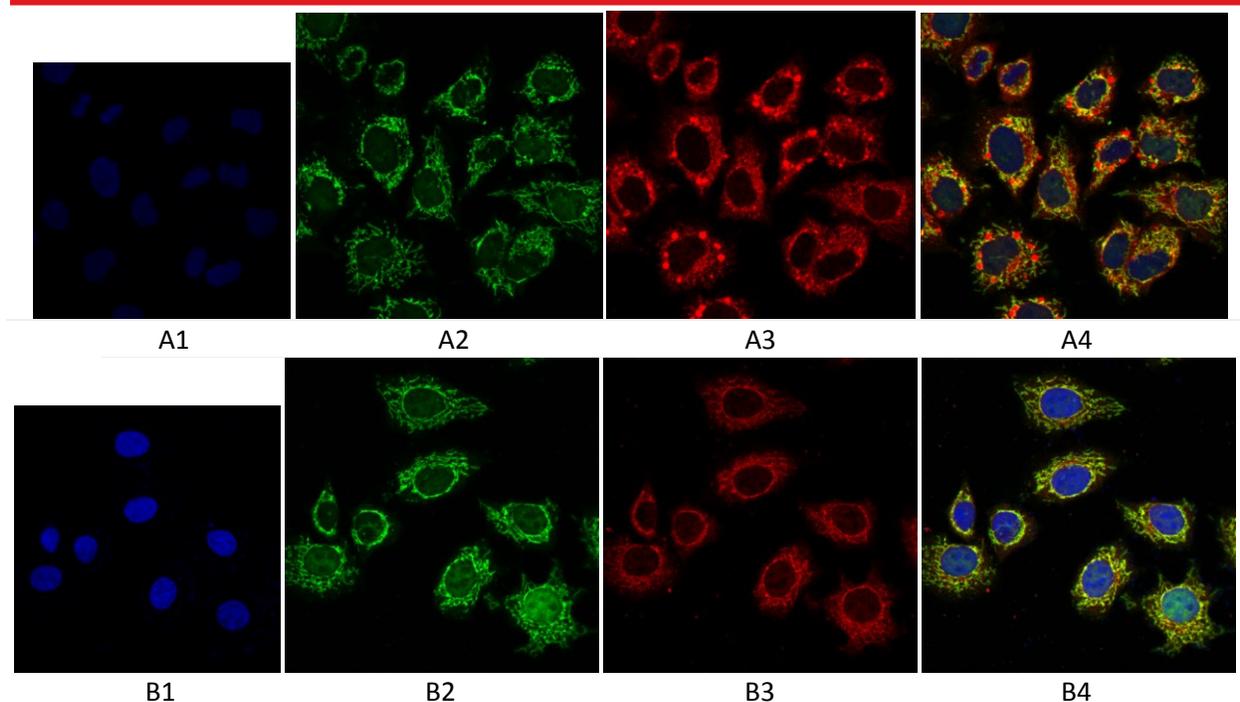


Figure 1 Immunofluorescence (IF) LSCM results of normal liver cells L02 cultured in RPMI 1640 with 10% for 40h (A1-A4) double stained with AF488-anti-SAH 839-6 (Cat# MAF00302) at 40 μ g/ml and R-PE-anti-SAM 84-3 (Cat# MAF00202) at 40 μ g/ml followed by DAPI staining. Hepatocellular carcinoma cell line HepG2 cells were cultured for 40h (B1-B4) and double stained with AF488-anti-SAH 839-6 (Cat# MAF00302) at 60 μ g/ml and R-PE-anti-SAM 84-3 (Cat# MAF00202) at 60 μ g/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. In this case, both SAM and SAH are seen more in cytoplasm (more in mitochondria areas) than nuclear.

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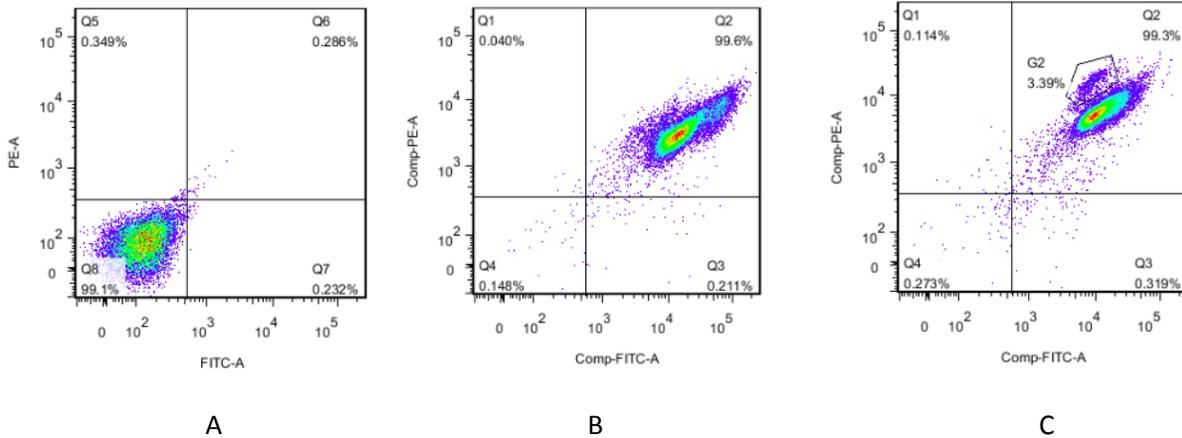


Figure 2 Flow Cytometry of L02 (B) and HepG2 (C) cells double stained with Alexa Fluoro 488 conjugated anti-SAH antibody 839-6 (Cat# MAF00302) at 45 $\mu\text{g/ml}$ and R-PE conjugated anti-SAM antibody 84-3 (Cat# MAF00202) at 45 $\mu\text{g/ml}$. 100% confluent cells (cultured in RPMI 1640 with 10% for 40h) were fixed and permeabilized with the nuclear fixation/permeabilization buffer (eBioscience 00-5523 FoxP3_TF Staining Buffer Set) and then double stained with antibodies indicated above. Cells were used for analysis with BD FACSCanto II Flow Cytometer. Both SAM and SAH are expressed ubiquitously yet rather dynamically. A: blank.

Hemans Chou
 Quality Control Team

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