

Certificate of Analysis

Anti-Phosphatidylserine, clone 1H6, Alexa Fluor[®] 488 conjugate

(mouse monoclonal IgG)

Catalog # 16-256

Lot # 41000

Immunogen: Liposomes containing 70% phosphatidylserine and 30% phosphatidylglycerol.

Specificity: Recognizes phosphatidylserine (PS) in cell membranes.

Applications: Flow cytometry.

Formulation: 100µg Alexa Fluor[®] 488 conjugated protein G purified mouse IgG in 200µl of PBS containing 1% BSA, 0.05% Tween, 0.05% sodium azide. Liquid at 4°C.

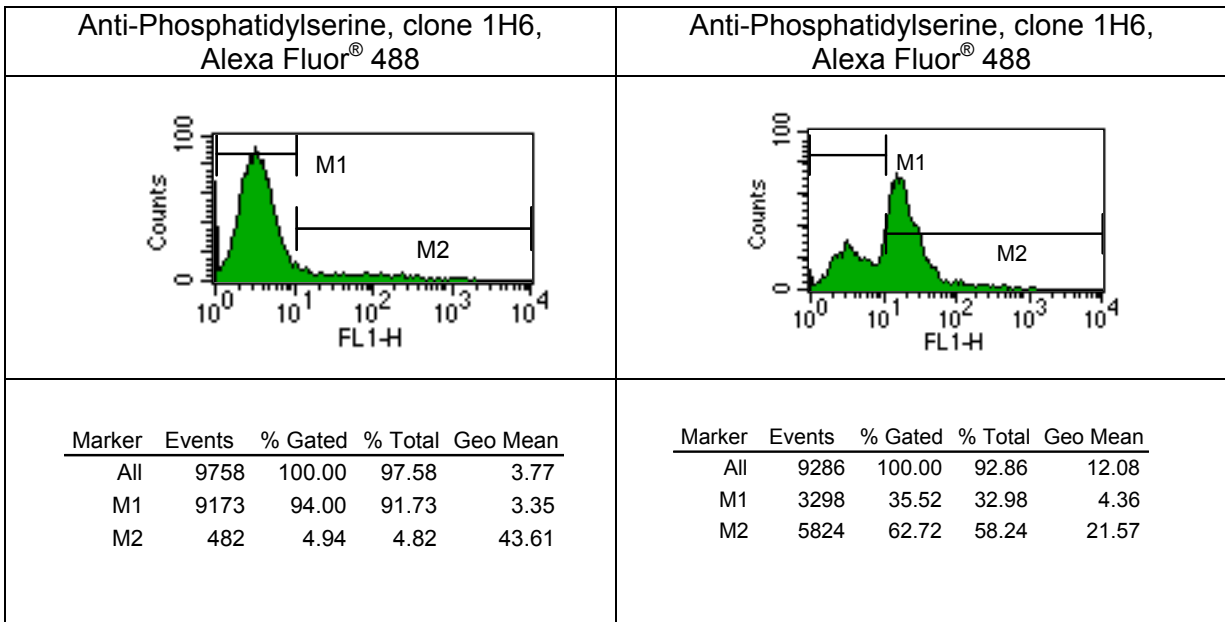
Storage and Stability: Do Not Freeze. Do not store the material diluted. Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing cap.

**FOR RESEARCH USE ONLY
 NOT FOR USE IN HUMANS**

Quality Control Testing

Flow Cytometry: 0.2µg of this lot detected Phosphatidylserine in fixed Jurkat cells.

Included Negative Control: Catalog # 16-240, Alexa Fluor[®] 488-conjugated Normal Mouse IgG.



Left panel: Jurkat cells stained with anti-Phosphatidylserine, clone 1H6, Alexa Fluor[®] 488 as a negative control (T=0).

Right panel: Jurkat cells stained with anti-Phosphatidylserine, clone 1H6, Alexa Fluor[®] 488 after pre-treatment with 2µg/ml Staurosporin for 8 hrs at 37°C.

Flow Cytometry Protocol

1. Harvest suspension cell lines by centrifugation. Count cells. You will need between 2×10^5 and 5×10^5 for each antibody test. Aliquot cells into 15ml conical test tubes.
2. Fix cells by adding 10% cell volume 37% methanol paraformaldehyde. Incubate 37°C for 10 min. Add 5ml PBS to cells. Invert to mix. Pellet cells.
3. Repeat 5ml PBS wash to remove remaining fixative.
4. Resuspend cells to 4×10^6 cells/ml staining buffer (1% FBS in PBS). Aliquot 50 μ l of cells (2.5×10^5 cells) per well of a 96 well round or V-bottom TC plate. Add 3.5 μ l of labeled antibody (60 μ g/ml antibody stock concentration = 0.21 μ g antibody per test well). Incubate on ice for one hour.
5. Add 150 μ l of PBS to each well to wash out unbound antibody. Centrifuge plate at 2000 rpm, 5 min, 4°C to pellet cells and remove supernatant.
6. Resuspend cell pellets in 150 μ l FACS buffer (4% FBS, 0.05% sodium azide, PBS). Transfer cell suspension to 1.2ml micro tube. Read on FACS machine using negative controls to set-up machine.
7. Number of cells per well and the amount of antibody used may need to be optimized for your individual application to give good, reproducible results. Note: Phosphatidylserine is membrane bound hence permeabilization of samples is not necessary.