



cell signaling solutions

Certificate of Analysis

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Anti-Phosphatidylserine, clone 1H6

(mouse monoclonal IgG)

Catalog # 05-719

Lot # 27684

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

Specificity: Recognizes phosphatidylserine (PS) in cell membranes.

Formulation: 200µg of protein G purified mouse IgG in 417µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

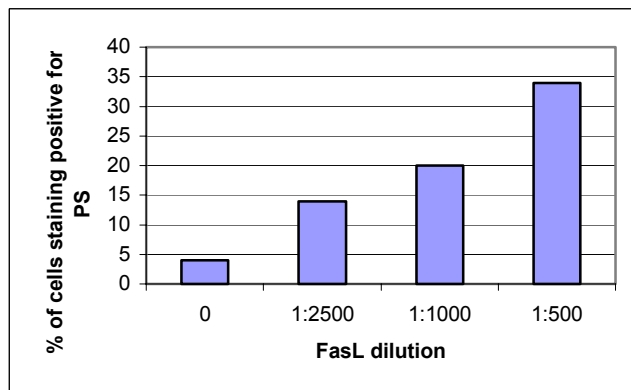
Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

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

Quality Control Testing

Flow Cytometry: 0.2-2µg/ml of this lot detected cell surface phosphatidylserine on Jurkat cells treated with Fas Ligand vesicles (Catalog # 01-210). See page two.

Immunohistochemistry: This antibody has been reported by an independent laboratory to detect phosphatidylserine in rat carotid artery paraffin sections fixed with ethanol/acetone.¹



Dose response of Fas Ligand using surface phosphatidylserine (PS) as readout:

Representative data from a previous lot. Jurkat cells were treated with increasing doses of Fas Ligand vesicles (Catalog # 01-210). After 18 hours, cells were surface stained with anti-phosphatidylserine antibody (2µg/ml), followed by a goat F(ab')₂ anti-mouse IgG phycoerythrin conjugate. Data was acquired and analyzed using a Guava[®] PCA-96 Express System.

Application Reference:

1. Mandinov, L., *et al.*, *Proc. Natl. Acad. Sci.* **100**: 6700-6705, 2003.

General References:

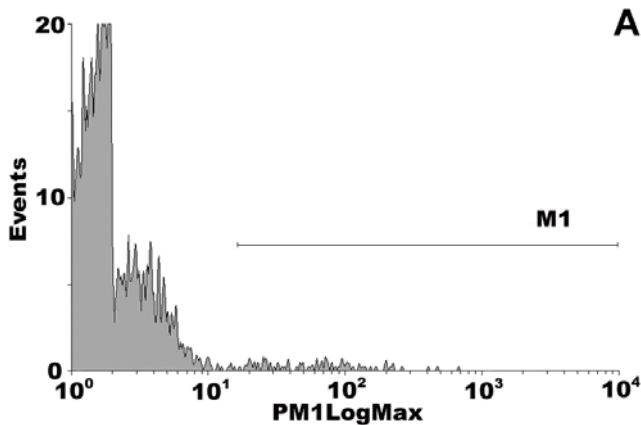
2. Koopman, G., *et al.*, *Blood* **84**: 1415-1420, 1994.
3. Vermes, I., *et al.*, *J. Immunol. Methods* **184**: 39-51, 1995.

FLOW CYTOMETRY PROTOCOL

(Adapted from the protocols provided with the Guava[®] PCA-96 System)

1. Suspend control and treated cells to 2×10^6 /ml per well in cold **PBSB** (PBS, 2% BSA, 0.05% sodium azide) and dispense 50 μ l of the cell suspension into each well of a 96-well microtiter plate.
2. Add 50 μ l anti-Phosphatidylserine antibody (Catalog # 05-719) in PBSB* to each well containing cells and incubate on ice for 20-30 minutes.
 - *a. Two-fold serial dilutions in the range of 0.2-2 μ g/ml (final concentration in presence of cells) are suggested in preliminary experiments to determine optimal conditions.
 - b. Include control wells of no primary antibody and normal mouse IgG (Catalog # 12-371) at 1 μ g/ml.
3. To each well, add 150 μ l cold PBSB and pellet cells by centrifugation at 300 x g for 5 minutes
4. Carefully remove supernatant and resuspend cells in 50 μ l of a diluted solution of an appropriate anti-mouse secondary fluorochrome-conjugate. (A 1:5 dilution of goat anti-mouse F(ab') IgG conjugated to phycoerythrin diluted in PBSB was used).
5. Protect from light for remainder of protocol. Incubate for 20-30 minutes on ice.
6. Wash cells as in step 3 above.
7. Carefully remove the supernatant and resuspend the cells in 200 μ l cold PBSB. Proceed with analysis/data acquisition of the stained cells using a flow cytometry instrument of choice.

Technical Note: This protocol can be performed in tubes rather than in a 96-well plate format.



Flow Cytometry:

Representative data from a previous lot. Jurkat cells were cultured for 18hr without (panel A) or with (panels B, C) Fas Ligand vesicles (1:500 dilution, Catalog # 01-210), then surface stained with anti-Phosphatidylserine at 0.2 μ g/ml (panels A, C) or a normal mouse IgG (Catalog # 12-371) at 1 μ g/ml (panel B), followed by a goat F(ab')₂ anti-mouse IgG phycoerythrin conjugate. Data was acquired and analyzed using a Guava[®] PCA-96 Express System.

