

# Anti-Phosphatidylserine, clone 1H6

Monoclonal Antibody

Cat. # 05-719

Lot # 28897

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY



## Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
FC, IH	Vrt	IgG	N/A	M	N/A	N/A

### Background

Phosphatidylserine (PS) is a phospholipid (1,2-diacyl-sn-glycerol-(3)-L-phosphoserine) found in the membrane of all plants and animals. PS is most concentrated in the brain, as PS makes up about 10% of the lipid content of neurons. Neural PS is thought to play a role in maintaining nerve cell integrity, enhancing neurotransmitter signal efficiency, and improving nerve cell signal transmission. One of the key features of apoptosis is the expression of PS on the outer cell membrane. PS is usually found on the inner side of the plasma membrane, but during early stage apoptosis, the PS is translocated to the outer membrane. FITC-labeled annexin V can bind to the exposed PS, providing the basis of most annexin V-based apoptosis assays.

### Presentation

Purified mouse monoclonal IgG in buffer containing 0.07M Tris-glycine, pH 7.4, 0.105M NaCl, 0.035% sodium azide and 30% glycerol. Liquid at -20°C.

### Concentration

1 mg/mL

### Specificity

Recognizes phosphatidylserine (PS) in cell membranes

### Immunogen

Liposomes containing 70% phosphatidylserine and 30% phosphatidylglycerol

### Method of Purification

Protein G Purified

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into

microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

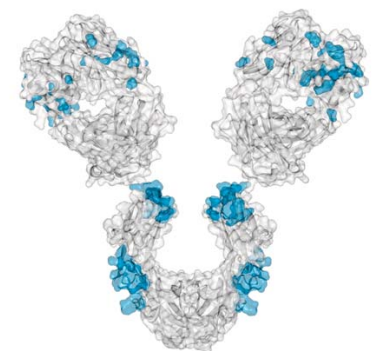
### Control

Brain tissue

### Research Applications

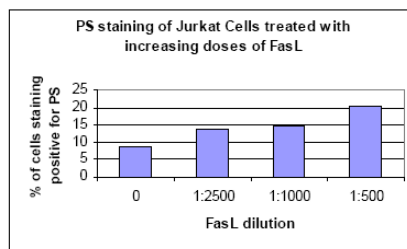
**Flow Cytometry:** 0.2-2 µg/mL of a previous lot detected cell surface phosphatidylserine on Jurkat cells treated with Fas Ligand vesicles (Catalog # 01-210). See page two.

**Immunohistochemistry:** This antibody has been reported by an independent laboratory to detect phosphatidylserine in rat carotid artery paraffin sections fixed with ethanol/acetone (Mandinov, L., 2003).



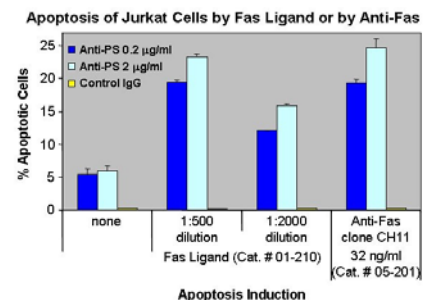
### References

- Vance, J.E., and Steenbergen, R. (2005). *Prog. Lipid Res.* 44: 207-234.
- Botto, M. (2004). *Arthritis Res. Ther.*, 6: 147-150.
- Kuypers, F.A., and de Jong, K. (2004). *Cell. Mol. Biol. (Noisy-le-grand)*. 50: 147-158.
- Nusbaum, P., et al. (2004). *Biochem. Soc. Trans.* 32: 477-479.
- Mandinov, L., et al. (2003). *Proc. Natl. Acad. Sci.* 100: 6700-6705.



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

Representative lot data. 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 (2 µg/mL), followed by a goat F(ab')<sub>2</sub> anti-mouse IgG phycoerythrin conjugate. Data was acquired and analyzed using a Guava® PCA-96 Express System.



Representative lot data. Anti-Phosphatidylserine, clone 1H6, was used at two concentrations (as indicated) to quantitate apoptosis induced in Jurkat cells by either Fas Ligand or by Anti-Fas, clone CH11, as indicated.

**APPLICATION LEGEND:** WB Western Blotting FC Flow Cytometry (FACS) IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit Vrt Vertebrates

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**PROTOCOL**

**FLOW CYTOMETRY**

(Adapted from the protocols provided with the Guava<sup>®</sup> PCA-96 System)

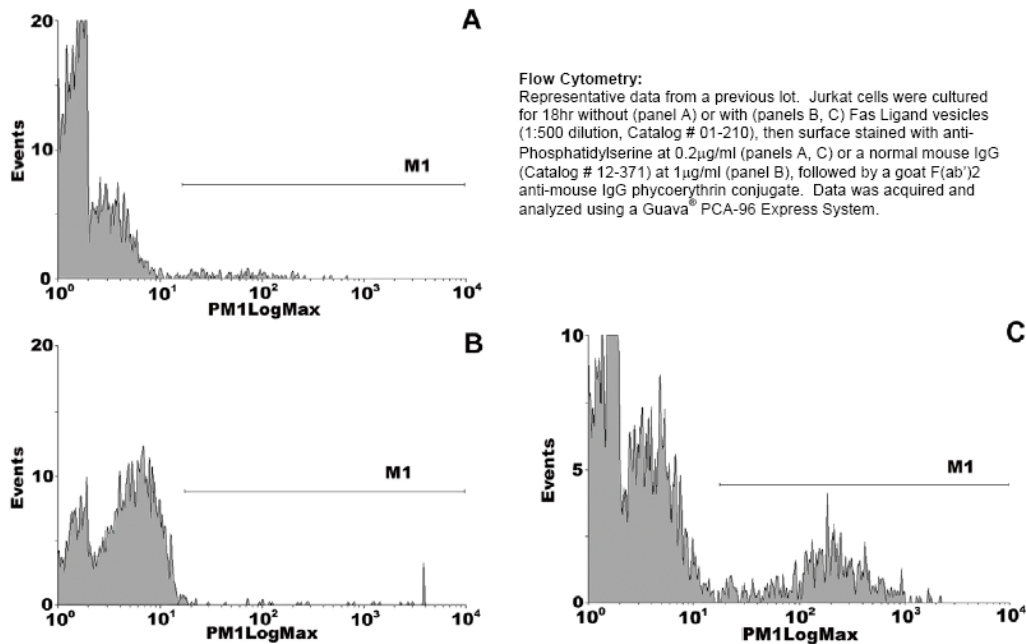
1. Suspend control and treated cells to 2 x 10<sup>6</sup>/mL per well in cold PBSB (PBS, 2% BSA, 0.05% sodium azide) and dispense 50 µL of the cell suspension into each well of a 96-well microtiter plate.
2. Add 50 µL anti-Phosphatidylserine antibody (Catalog # 05-719) in PBSB\* to each well containing cells and incubate on ice for 20-30 minutes.

\*a. Two-fold serial dilutions in the range of 0.2-2 µg/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 µg/mL.

3. To each well, add 150 µL cold PBSB and pellet cells by centrifugation at 300 x g for 5 minutes
4. Carefully remove supernatant and resuspend cells in 50µl 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 µL 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.



**RELATED PRODUCTS (specific)**

cat #	description
16-256	■ Anti-Phosphatidylserine, clone 1H6, Alexa Fluor <sup>®</sup> 488 conjugate
12-349	■ Goat Anti-Mouse IgG, HRP conjugate

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow <sup>™</sup> ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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