

**PERFORMANCE DATA SHEET**

3141

**Monoclonal anti-human Perforin/APC\***

**mAb name/Clone:**  $\delta$ G9

**Isotype:** Mouse IgG2b kappa

**Immunogen:** Purified granules from human YT lymphoma cell line

**CATALOG#:** 358-050

**QUANTITY:** 120 tests

**VOLUME IN VIAL:** 0.2 ml

**WORKING DILUTION:** 1:50 (or use 1.6 $\mu$ l of concentrated reagent per 5x10<sup>5</sup>-cell test)

**INFORMATION:** Human perforin (cytolysin) is believed to be one of the major effector molecules used by cytotoxic T cells and NK cells to mediate targeted cell lysis. Perforin expression is constitutive on NK cells, but increases in resting CD8<sup>+</sup> cytotoxic cells upon activation. Antibody  $\delta$ G9 recognizes the human perforin molecule of 70 kd.

**References:** M.J. Smyth, et al. (1990) J Exp Med **171**: 1269-1281. A. Hameed, et al, Am J Pathol (1992) **140**: 1025-1030. P.L. Jose, et al, (1994) J Immunol **148**: 3354-3360. B.C. Schlesinger & L. Cheng, Immunol (1994) **81**: 291-295. C.C. Liu, et al, (1995) Immunol Today **16**: 194-201. A.H. Hombach, H Abken, et al. (2006) J Immunol **177**: 5668-5675.

**STORAGE CONDITIONS:** Store at 2 - 5°C. Do not freeze! Protect from light.

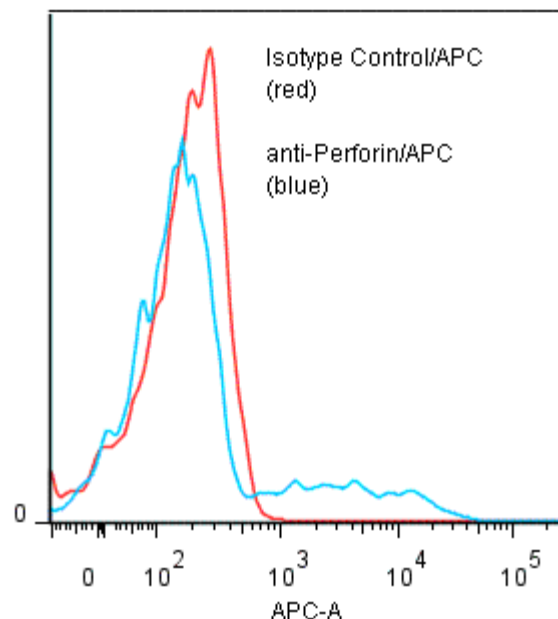
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: \_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (as a preservative).

**PRODUCTION:** Protein A purified antibody from tissue culture supernatant was conjugated to cross-linked Allophycocyanin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at approximately **0.25 mg/ml** with an APC : mAb molar ratio of 1.13 .

**PERFORMANCE:** Ficoll prepared human **peripheral blood mononuclear cells** were stimulated by incubating 2 days at 5 x 10<sup>6</sup> cells/ml in RPMI 10% FBS media including 5  $\mu$ g/ml Phytohemagglutinin-P. Cells were then fixed for 30 min with 2% buffered formaldehyde after which they were washed two times. Subsequent reagent incubations and washes were done using a buffer containing **0.3% Saponin** to permeabilize cells. Five x 10<sup>5</sup> cells per tube were washed and incubated 45 minutes on ice with 80  $\mu$ l of anti-perforin/APC at a **1:50** dilution (5  $\mu$ g/ml). Cells were washed three times, fixed and analyzed by FACS. A net **16.1%** sub population of the cells shifted positively with a mean shift of **1.49** log<sub>10</sub> fluorescent units when compared to an IgG2b/R-PE isotype Control (cat #284-060). Binding was blocked when cells were pre incubated 10 minutes with 20  $\mu$ l of 0.5 mg/ml unlabeled anti-perforin antibody (Catalog #358-020).

**Binding of anti-Perforin/APC to fixed permeabilized stimulated human PBL**



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