

PERFORMANCE DATA SHEET

1818

*Monoclonal anti-human perforin/R-PE**

mAb name/Clone: δ 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 μ l of concentrated reagent per 5x10⁵-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⁺ cytotoxic cells upon activation. Antibody δ 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% NaN₃ (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at 0.5 mg/ml.

PERFORMANCE: Ficoll prepared human peripheral blood mononuclear cells were stimulated by incubating 2 days at 5 x 10⁶ cells/ml in RPMI 10% FBS media including 5 μ 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 0.5% Tween 20 to permeabilize cells. Five x 10⁵ cells per tube were washed and incubated 45 minutes on ice with 80 μ l of anti-perforin/R-PE at a 1:50 dilution (10 μ g/ml). Cells were washed three times, fixed and analyzed by FACS. A net 12% population of the cells shifted positively with a mean shift of 1.9 log₁₀ fluorescent units when compared to an IgG2b/R-PE isotype Control (cat #284-050) at a similar concentration. Binding was blocked when cells were pre incubated 10 minutes with 20 μ l of 0.5 mg/ml unlabeled anti-perforin antibody (Catalog #358-020).

**This Product is intended for Laboratory Research use only.* R-Phycoerythrin (R-PE) is covered under patents: U.S. 4,520,110; European 76,695 and Canadian 1,179,942.

Binding of anti-perforin/PE to fixed permeabilized stimulated human PBMC

