

PERFORMANCE DATA SHEET

1818

Monoclonal anti-human perforin/FITC*

mAb name/Clone: delta G9

Isotype: Mouse IgG2b kappa

Immunogen: Purified granules from human YT lymphoma cell line

CATALOG#: 358-040

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2 ml

WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5 x 10⁵-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 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. Freeze/Thawing is not recommended. Protect from light.

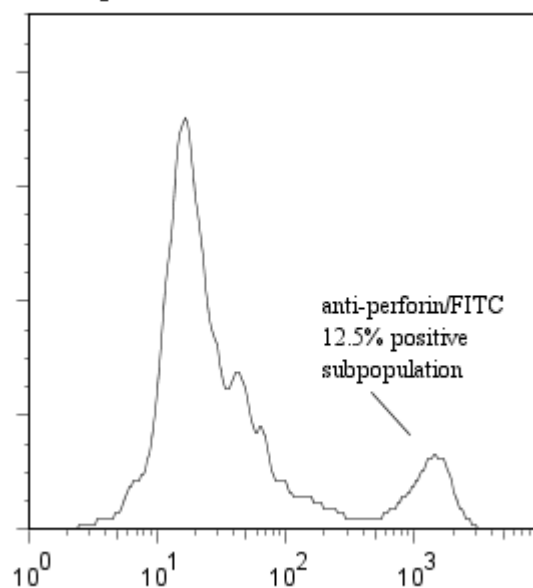
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: _____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

PRODUCTION: Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to 95% Immunoglobulin by SDS-PAGE, and reacted with FITC. Unconjugated FITC was removed from conjugate using a desalting column. Antibody conjugate is at a concentration of 0.1 mg/ml.

PERFORMANCE: Ficoll prepared human peripheral blood mononuclear cells were stimulated 1 day by incubating 5 x 10⁶ cells/ml in RPMI 10% FBS media containing 5 µg/ml Phytohemagglutinin-P. Cells were then collected and fixed for 30 min with 2% buffered formaldehyde and washed two times with buffer. Cells were permeabilized using a buffer containing 0.3% Saponin, washed and pre incubated 10 minutes with 20 µl human IgG at 250 µg/ml (to block non specific binding) and then incubated 45 minutes on ice with 80 µl of anti-perforin/FITC at a 1:50 dilution (2 µg/ml). Cells were washed three times, fixed and analyzed by FACS. A net 12.5% sub population of the cells stained positive with a mean shift of 1.2 log₁₀ fluorescent units when compared to a Mouse IgG2b/FITC negative control (Catalog #284-040). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-perforin antibody (Catalog #358-020).

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



* Research use only. Not for use in Diagnostic procedures.