**PERFORMANCE DATA SHEET**
**1850**

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

***mAb name/Clone:*** **δG9**
***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 1.6µl of concentrated reagent per $5x10^5$-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 δG9 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.***

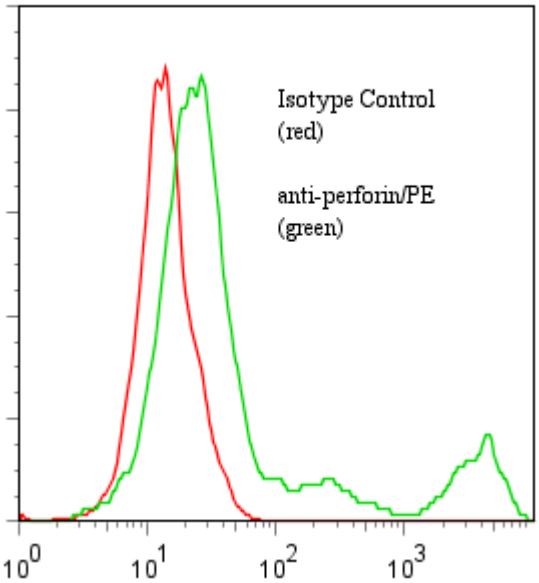
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% $NaN_3$ (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 $10^6$ cells/ml in RPMI 10% FBS media including 5 µg/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 **0.1% Tween 20** to permeabolize cells. Five x $10^5$ cells per tube were washed and incubated 45 minutes on ice with 80 µl of anti-perforin/R-PE at a **1:50** dilution (10µg/ml). Cells were washed three times, fixed and analyzed by FACS. A net **12%** sub population of the cells shifted positively with a mean shift of **1. 9** $\log_{10}$ fluorescent units when compared to an IgG2b/R-PE isotype Control (cat #284-050). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml unlabeled anti-perforin antibody (Catalog #358-020).

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

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**Ancell Corporation P.O. Box 87 Bayport, MN 55003-0087 USA**
**Phone: Toll free 800-374-9523 or 651-439-0835 Fax: 651-439-1940**