# Hu-CD56/CD69/CD4 3 Color FCM Reagent: *sc-3608*



### BACKGROUND

Human CD56/CD69/CD4 sc-3608 is a direct immunofluorescence reagent formatted to identify NK- (CD56+) lymphocyte activation in peripheral blood. CD56 is present on essentially all resting and activated CD16+ natural killer (NK) cells and on a small percentage of CD3+ peripheral blood lymphocytes (1). CD56 expression decreases when NK cells are activated (1). CD3+CD56+ T lymphocytes comprise a subset of cytotoxic T lymphocytes that mediates non-MHC restricted cytotoxicity (2). In normal peripheral blood, CD69 is variably expressed on lymphocytes (3). Upon activation, CD69 expression increases on T, B, and NK lymphocytes (4). In thymus, CD69 is constitutively expressed on the bright CD3+ subset of T cells, mostly on subpopulations of CD4+ CD8- or CD4- CD8+ T cells (4). CD4 identifies helper/inducer T lymphocytes and binds class II MHC molecules (5). CD4 is also the primary receptor for HIV (6).

Antigen Expression	Cell Type Identified	
CD4+	Helper/Inducer T Cells	
CD4+ CD69+	Activated Helper/Inducer T Cells	

### STORAGE

Store at 4° C. Do not freeze. Stable for one year from the date of shipment. Protect reagents from prolonged exposure to light.

### PRODUCT

Supplied in 1.0 ml of PBS containing 0.1% azide and 0.1% gelatin. Sufficient for 50 tests. This product has been titrated for optimal performance. Recommended use is 20 uL per test ( $1x10^6$  cells). For research use only. Not for use in diagnostic procedures.

#### INSTRUMENT

Human CD56/CD69/CD4 sc-3608 is recommended for use with either a single or dual laser Flow Cytometer fitted with appropriate acquisition and analysis software, such as the FACSCalibur™ Flow Cytometer fitted with CellQuest<sup>™</sup> Software by Becton Dickinson.

The flow cytometer must be equipped with a 488 nm laser and must be capable of detecting light scatter (forward and side) and three-color fluorescence with emission detectable in three ranges: 515-545 nm, 562-607 nm and >650 nm, and it must be able to threshold and discriminate using the >650 channel.

Antigen	Clone	Isotype	Label*	Detection Range (nm)
CD56	123C3	IgG <sub>1</sub>	FITC	515-545
CD69	FN50	IgG <sub>1</sub>	PE	562-607
CD4	2D-1	IgG1	PE-Cy5	>650

\*Fluorescent labels include FITC: Fluorescein isothiocyanate; PE: phycoerythrin; PE-Cy5: phycoerythrin-cyanin 5.

## **ISOTYPE CONTROL**

sc-2940 CON  $(IgG_1 FITC/IgG_1 PE/IgG_1 PE-Cy5)$  is the isotype matched negative control for this system and is suitable for 50 tests.

#### REFERENCES

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3. Schwarting, R., Biedobitek, G., and Stein, H. Cluster report: CD69. Knapp, W., Dörken, B., Gilks, W.R., *et al.* eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989: 428-432.

4. Testi, R., Phillips, J.H., and Lanier, L.L. 1988. Constitutive expression of a phosphorylated activation antigen (Leu 23) by CD3bright human thymocytes. J. Immunol. <u>141</u>: 2557-2563.

5. Gallagher, P.F., Fazekas de St. Groth, B., and Miller, J.F. 1989. CD4 and CD8 molecues can physically associate with the same T-cell receptor. Proc. Natl. Acad. Sci. USA <u>86</u>: 10044-10048.

6. Dalgleish, A.G., Beverley, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F., and Weiss, R.A. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature <u>312</u>: 763-767.