Mo-TNFα/CD69/CD4 3 Color FCM Reagent: *sc-3619*



BACKGROUND

Mouse $TNF\alpha/CD69/CD4$: sc-3619 is a immunofluorescence reagent formatted to detect intracellular cytokines and the early activation marker CD69 in antigenactivated CD4+ T lymphocytes in whole blood. TNFa is a pleiotropic cytokine that mediates inflammatory, immunological, and pathophysiological reactions (1). CD4+ and CD8+ T lymphocytes rapidly produce TNFa upon stimulation by phorbol diester and calcium ionophore (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 the helper/inducer T lymphocyte subset that is present on normal peripheral blood lymphocytes (5,6). CD4 binds class II MHC molecules (6) and is the primary receptor for HIV (7).

Antigen Expression	Cell Type Identified	
CD3+ CD4+	Helper/Inducer T Cells	
CD3+ CD4+ CD8- CD69+	Activated Thymocytes	

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 (1x10⁶ cells). **For research use only. Not for use in diagnostic procedures.**

INSTRUMENT

Mouse TNF α /CD69/CD4: sc-3619 is recommended for use with a dual laser Flow Cytometer fitted with appropriate acquisition and analysis software, such as the FACSCalibur[™] Flow Cytometer fitted with CellQuest[™] Software by Becton Dickinson.

The flow cytometer must be equipped with 635 nm and 488 nm lasers and must be capable of detecting light scatter (forward and side) and four-color fluorescence with emission detectable in four ranges: 515-545 nm, 562-607 nm, >650 nm and 652-668 nm, and it must be able to threshold and discriminate using the >650 channel.

Antigen	Clone	Isotype	Label*	Detection Range (nm)
TNFα	TN3-19.12	Armenian Hamster IgG	FITC	515-545
CD69	H1.2F3	Armenian Hamster IgG	PE	562-607
CD4	H129.19	rat IgG _{2a}	PE-Cy5	>650

^{*}Fluorescent labels include FITC: Fluorescein isothiocyanate; PE: phycoerythrin; PE-Cy5: phycoerythrin-cyanin 5; APC: allophycocyanin

ISOTYPE CONTROL

sc-3619 CON (Armenian Hamster IgG FITC/Armenian Hamster IgG PE/rat Ig G_{2a} PE-Cy5) is the isotype matched negative control for this system and is suitable for 50 tests.

REFERENCES

- 1. Old, L. 1985. Tumor Necrosis Factor (TNF). Science 230: 630-632.
- 2. Cuturi, M.C., Murphy, M., Costa-Giomi, M.P., Weinmann, R., Perussia, B., and Trinchieri, G. 1987. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. J. Exp. Med. 165: 1581-1594.
- 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. 141: 2557-2563.
- 5. Reichert, T., DeBruyere, M., Deneys, V., Totterman, T., Lydyard, P., Yuksel, F., Chapel, H., Jewell, D., Van Hove, L., Linden, J., *et al.* 1991. Lymphocyte subset reference ranges in adult Caucasians. Clin. Immunol. Immunopathol. <u>60</u>: 190-208.
- 6. 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 86: 10044-10048.
- 7. 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 312: 763-767.