Mo-CD8/CD28 2 Color FCM Reagent: *sc-3982*



BACKGROUND

Mouse CD8/CD28: sc-3982 is a direct immunofluorescence reagent formatted to identify and determine the percentage of activated suppressor/cytotoxic T lymphocytes in erythrocyte-lysed whole blood, based on cell-surface antigen expression. CD8 identifies suppressor/cytotoxic T lymphocytes (1,2) and binds class I MHC molecules, resulting in enhanced activation of resting T lymphocytes (3). CD28 is a ligand for the B7/BB1 antigen and is expressed on most T lymphocytes (4). CD28 expression increases during thymocyte maturation and in response to cell activation (4). CD8+CD28+ lymphocytes mediate alloantigen-specific cytotoxicity that is major histocompatibility complex (MHC) class I-restricted. CD8+CD28- lymphocytes mediate suppression of cell proliferation (4-6). T cells from both HIV+ and aged individuals show an increased number of CD8+ CD28- T cells (7).

Antigen Expression	Cell Type Identified	
CD8+	Suppressor/Cytotoxic T Cells	
CD8+ CD28+	Mediate Cytotoxicity	
CD8+ CD28-	Suppress Cell Proliferation	

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 CD8/CD28: sc-3982 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™ 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 two-color fluorescence with emission detectable in two ranges: 515-545 nm, 562-607 nm.

Antigen	Clone	Isotype	Label*	Detection Range (nm)
CD8	53-6.7	rat IgG _{2a}	FITC	515-545
CD28	37.51.1	Syrian Hamster IgG	PE	562-607

^{*}Fluorescent labels include FITC: Fluorescein isothiocyanate; PE: phycoerythrin

ISOTYPE CONTROL

sc-3982 CON (rat ${\rm IgG_{2a}}$ FITC/Syrian hamster IgG PE) is the isotype matched negative control for this system and is suitable for 50 tests.

REFERENCES

- 1. Ledbetter, J.A., Evans, R.L., Lipinski, M., Cunningham-Rundles, C., Good, R.A., and Herzenberg, L.A. 1981. Evolutionary conservation of surface molecules that distinguish T-lymphocyte helper/inducer and T cytotoxic/suppressor subpopulations in mouse and man. J. Exp. Med. 153: 310-323.
- 2. Evans, R.L., Wall, D.W., Platsoucas, C.D., *et al.* 1981. Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to the TH2 antigen. Proc. Natl. Acad. Sci. USA <u>78</u>: 544-548.
- 3. 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.
- 4. June, C.H., Ledbetter, J.A., Linsley, P.S., and Thompson, C.B. 1990. Role of the CD28 receptor in T-cell activation. Immunol. Today 11: 211-6.
- 5. Lum, L.G., Orcutt-Thordarson, N., Seigneuret, M.C., and Hansen, J.A. 1982. *In vitro* regulation of immunoglobulin synthesis by T-cell subpopulations defined by a new human T-cell antigen (9.3). Cell Immunol. <u>72</u>: 122-129.
- 6. Damle, N.K., Mohagheghpour, N., Hansen, J.A., and Engleman, E.G. 1983. Alloantigen-specific cytotoxic and suppressor T lymphocytes are derived from phenotypically distinct precursors. J. Immunol. 131: 2296-2300.
- 7. Eylar, E.H., Lefranc, C.E., Yamamura, Y., Baez, I., Colon-Martinez, S.L., Rodriguez, N., and Breithaupt, T.B. 2001. HIV infection and aging: enhanced Interferon- and Tumor Necrosis Factor-alpha production by the CD8+ CD28- T subset. BMC Immunol. <u>2</u>: 10.