# Mo-CD3/CD8/CD45/CD4 4 Color FCM Reagent: *sc-3964*



## BACKGROUND

Mouse CD3/CD8/CD45/CD4: sc-3964 is a direct immunofluorescence reagent formatted to identify and determine the percentage of mature T cells, suppressor/cytotoxic T cells and helper/inducer T cells in erythrocyte-lysed whole blood, based on cell-surface antigen expression. CD3 identifies T lymphocytes and non-covalently associates with either  $\alpha/\beta$  or  $\gamma/\delta$  TCR, which recognizes antigens associated with the MHC (1). CD8 identifies suppressor/cytotoxic T lymphocytes and binds class I MHC molecules, which enhances the activation of resting T lymphocytes (2). CD45 is a major leukocyte cell surface molecule that plays a role in pre-TCR signal transduction (3,4). The pre-TCR complex regulates the transition from CD4-CD8- double-negative to CD4+CD8+ double-positive thymocytes during T cell development (4). CD4 identifies helper/inducer T lymphocytes and binds class II MHC molecules (2). CD3+CD8+ and CD3+CD4+ percentages or counts are used to characterize and monitor some forms of immunodeficiency and autoimmune disease (5,6).

Antigen Expression	Cell Type Identified
CD3+	Mature T Cells
CD3+ CD8+	Suppressor/Cytotoxic T Cells
CD3+ CD4+	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

Mouse CD3/CD8/CD45/CD4: sc-3964 is recommended for use with a dual laser Flow Cytometer fitted with appropriate acquisition and analysis software, such as the FACSCalibur<sup>™</sup> Flow Cytometer fitted with CellQuest<sup>™</sup> 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)
CD3	145-2C11	Armenian Hamster IgG	FITC	515-545
CD8	53-6.7	rat IgG <sub>2a</sub>	PE	562-607
CD45	MB4B4	rat IgG	PE-Cy5	>650
CD4	H129.19	rat IgG <sub>2a</sub>	APC	652-668

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

#### **ISOTYPE CONTROL**

sc-3964 CON (Armenian Hamster IgG FITC/rat IgG<sub>2a</sub> PE/rat IgG PE-Cy5/rat IgG<sub>2a</sub> APC) is the isotype matched negative control for this system and is suitable for 50 tests.

#### REFERENCES

1. Exley, M., Terhorst, C., and Wileman, T. 1991. Structure, assembly and intracellular transport of the T cell receptor for antigen. Semin. Immunol. <u>3</u>: 283-297.

2. 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.

3. Charbonneau, H., Tonks, N.K., Walsh, K.A., and Fischer, E.H. 1988. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. Proc. Natl. Acad. Sci. USA <u>85</u>: 7182-7186.

4. Pingel, S., Baker, M., Turner, M., Holmes, N., and Alexander, D.R. 1999. The CD45 tyrosine phosphatase regulates CD3-induced signal transduction and T cell development in recombinase-deficient mice: restoration of pre-TCR function by active p56(lck). Eur. J. Immunol. <u>29</u>: 2376-2384.

5. Foucar, K. and Goeken, J.A. 1982. Clinical Applications of immunologic techniques to the diagnosis of lymphoproliferative and immunodeficiency disorders. Lab. Med. <u>13</u>: 403-413.

6. Smolen, J.S., Chused, T.M., Leiserson, W.M., Reeves, J.P., Alling, D., and Steinberg, A.D. 1982. Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus. Correlation with clinical features. Am. J. Med. <u>2</u>: 783-790.