# Hu-CD45RA/CD45RO/CD3/CD4 4 Color FCM Reagent: *sc-2946*



# BACKGROUND

Human CD45RA/CD45RO/CD3/CD4: sc-2946 is a direct immunofluorescence reagent formatted to identify and determine the percentage of mature T cells and helper/inducer (naive and memory) T cells in erythrocyte-lysed whole blood, based on cell-surface antigen expression. CD45 is a major leukocyte cell surface molecule that is essential for the activation of T and B lymphocytes (1,2). In T cells, the alternative splicing of CD45 is regulated so that naive or unprimed T cells predominantly express CD45RA-positive isoforms and switch to expression of CD45RO upon activation. CD45RO expression is correlated with the memory T-cell phenotype (3). CD3 identifies T lymphocytes and non-covalently associates with either  $\alpha/\beta$  or  $\gamma/\delta$  TCR, which recognizes antigens associated with the MHC (4). CD4 identifies helper/inducer T lymphocytes and binds class II MHC molecules (5).

Antigen Expression	Cell Type Identified
CD3+	Mature T Cells
CD3+ CD4+	Helper/Inducer T Cells
CD3+ CD4+ CD45RA+	Helper/Inducer Naive T Cells
CD3+ CD4+ CD45RO+	Helper/Inducer Memory 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 CD45RA/CD45RO/CD3/CD4: sc-2946 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)
CD45RA	4KB5	IgG <sub>1</sub>	FITC	515-545
CD45RO	UCH-L1	IgG <sub>2a</sub>	PE	562-607
CD3	UCH-T1	IgG <sub>1</sub>	PE-Cy5	>650
CD4	MT310	IgG <sub>1</sub>	APC	652-668

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

#### **ISOTYPE CONTROL**

sc-2946 CON  $(IgG_1 FITC/IgG_{2a} PE/IgG_1 PE-Cy5/IgG_{2b} APC)$  is the isotype matched negative control for this system and is suitable for 50 tests.

### REFERENCES

1. 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.

2. Kishihara, K., Penninger, J., Wallace, V.A., Kundig, T.M., Kawai, K., Wakeham, A., Timms, E., Pfeffer, K., Ohashi, P.S., and Thomas, M.L. 1993. Normal lymphocyte development but impaired cell maturation in CD45-exon6 protein tyrosine phosphatase-deficient mice. Cell <u>74</u>: 143-156.

3. Akbar, A.N., Terry, L.; Timms, A., Beverley, P.C., and Janossy, G. 1988. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J. Immun. <u>140</u>: 2171-2178.

4. 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.

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.