Hu-IFN-γ/CD69/CD8/CD3 4 Color FCM Reagent: *sc-3603*



BACKGROUND

Human IFN-y/CD69/CD8/CD3: sc-3603 is a direct immunofluorescence reagent formatted to detect intracellular IFN-y and the activation marker CD69 in antigen-activated CD8+ T lymphocytes in erythrocyte-lysed whole blood. IFN- γ is a multifunctional immunomodulator with anti-tumor and anti-viral activity (1,2). Upon activation, IFN-y is produced by most CD8+ T lymphocytes, by the T_{H1} and T_{H0} subsets of CD4+ lymphocytes (1,2). 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). CD8 identifies suppressor/cytotoxic T lymphocytes and binds class I MHC molecules, which enhances the activation of resting T lymphocytes (5). CD3 identifies T lymphocytes and noncovalently associates with either α/β or γ/δ TCR, which recognizes antigens associated with the MHC (6). CD3+CD8+ and CD3+CD4+ counts are used to characterize and monitor some forms of immunodeficiency and autoimmune disease (7,8).

Antigen Expression	Cell Type Identified	
CD3+	Mature T Cells	
CD3+ CD8+	Suppressor/Cytotoxic 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 $(1x10^6 \text{ cells})$. For research use only. Not for use in diagnostic procedures.

INSTRUMENT

Human IFN-γ/CD69/CD8/CD3: sc-3603 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)
IFNγ	G-23	IgG ₁	FITC	515-545
CD69	FN50	IgG ₁	PE	562-607
CD8	HIT8a	IgG ₁	PE-Cy5	>650
CD3	UCH-T1	IgG ₁	APC	652-668

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

ISOTYPE CONTROL

sc-3603 CON (IgG_1 FITC/ IgG_1 PE/ IgG_1 PE- $Cy5/IgG_1$ APC) is the isotype matched negative control for this system and is suitable for 50 tests.

REFERENCES

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