A Rapid, High Throughput Multiplex Assay that Identifies T-cell Subsets and Measures T-Cell Activation and Cytokine Secretion

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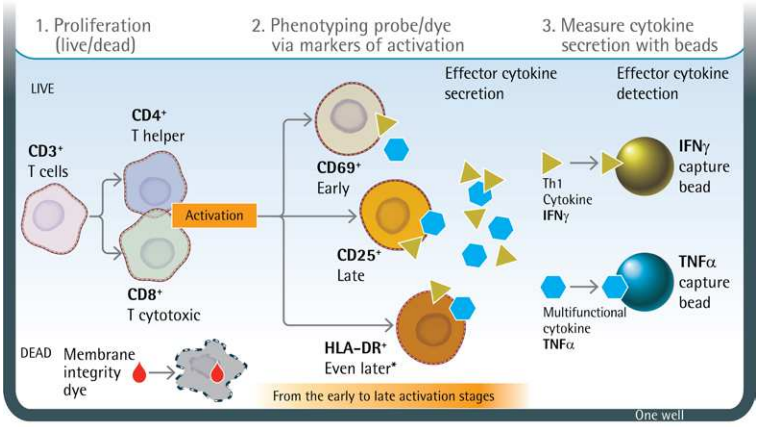
Abstract

T cells play a critical role in the adaptive immune response. In naive T cells, binding of the T cell receptor (TCR) to peptides complexed with major histocompatibility complex (MHC) triggers an intricate signaling mechanism leading to T cell activation, proliferation and production of cytokines. Modulating the TCR signaling pathway using biologics, small molecules or genetic engineering is highly relevant to many therapeutic areas including cancer immunotherapy, adoptive cell therapy, vaccine development and autoimmune diseases. The development of these drugs and therapies require the routine use of assays to profile T cell function and health.

To address the need for rapid monitoring of immune cell function, Intellicyt has developed an optimized, high throughput flow cytometry assay to measure T cell activation. The T Cell Activation Cell and Cytokine Profiling Kit greatly streamlines the traditional workflow by measuring cell phenotype, T cell activation markers, cell proliferation, cell viability and quantitates secreted cytokines in a single 10 ul sample using a miniaturized multi-well plate format

Assay Biochemistry



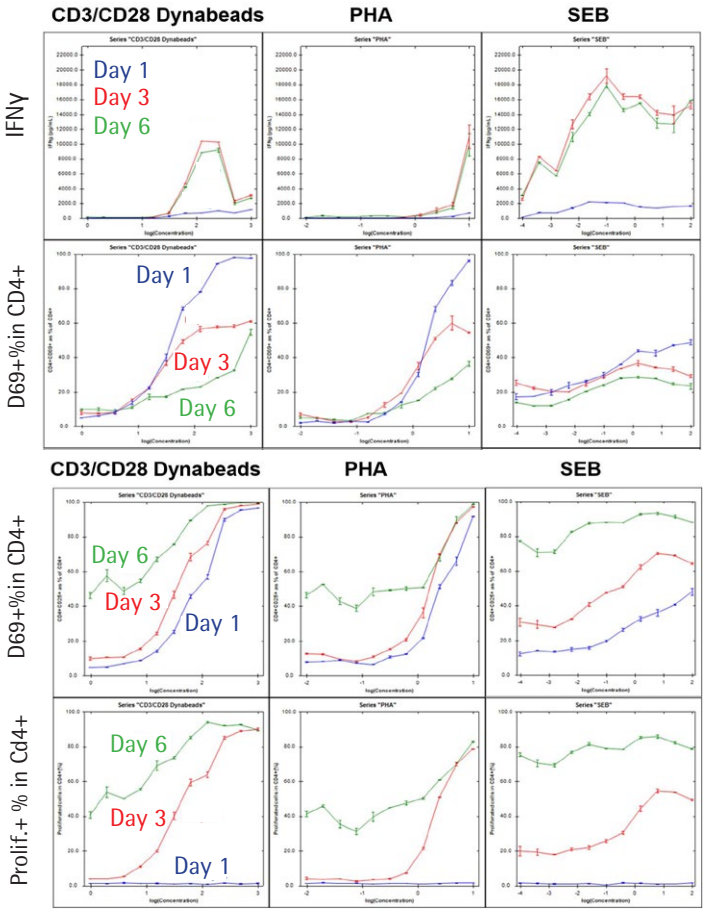
The assay discriminates between live and dead cells by using a membrane integrity dye which only stains dead cells. Live cells in each well are phenotyped by CD3, CD4 and CD8 antibodies to identify the various T cell subsets. Cell surface activation markers are measured to determine early activation (CD69+), late activation (CD25+) and even later activation (HLA-DR+) in the different T cell subpopulations. The levels of secreted IFNγ and TNFα are quantitated in the same sample well using a bead-based assay.

Results

PBMC's were stained with the proliferation and encoder dye and were stimulated with 3 different T Cell activators (CD3/28 Dynabeads, PHA or Staphylococcal enterotoxin B) using a 12 point, 2 fold serial dilution series. On days 1, 3 and 6 after stimulation, 10 ul of samples containing cells and supernatant were transferred to an assay plate and analyzed using the T Cell Activation Cell and Cytokine Profiling Kit.

Data were acquired on the iQue Screener PLUS and analyzed using the integrated ForeCyt software. The data in Figure 1 shows dose and temporal responses in the percentage of activated T Cells and the amount of secreted IFNγ among the different treatments. Furthermore, unique patterns of T Cell activation markers were observed with each compounds.

Figure 1: Representative T Cell Activation Data



Summary

The Human T Cell Activation Cell and Cytokine Profiling kit is an optimized, high throughput, multiplexed assay which provides rapid and routine monitoring of in vitro T-cell activation/proliferation. The assay uses only 5-10 ul of sample, saving precious cells and reagents and the sample acquisition and analysis time for a 96 well plate when run on the iQue Screener PLUS platform is 15 minutes. High content data is provided by the integrated software and assay template which auto generates all cell and bead gates, cell metrics, IC50 and EC50 curves, and quantitates secreted cytokine levels.

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