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Rapid, High Capacity Monitoring of T Cell Activation for Adoptive Cell Therapy

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Abstract

Monitoring the *ex vivo* activation of human T lymphocytes is key to developing an optimized and scalable adoptive cell therapy process. Described here is the development of a large-scale, multiplexed assay using high throughput flow cytometry to assess T cell activation over time by combining immunophenotyping, cell proliferation, and cytokine profiling in a single assay.

To address the need for rapid monitoring of immune cell function, Sartorius has developed an optimized, high content, multiplexed assay using high throughput flow cytometry to measure T cell activation. The iQue® T Cell Activation 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 sample using a miniaturized multi-well plate format.

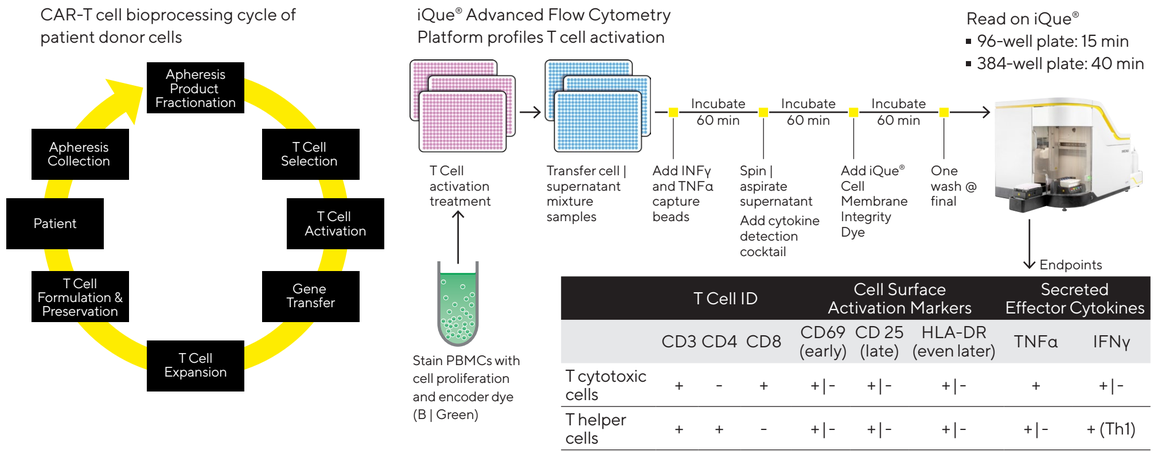
As a proof of concept, PBMCs were stained with cell proliferation and encoding dye and were stimulated with three different T cell activation compounds, using a 12-point dose response series. On days 1, 3, and 6 after stimulation, 10 mL of cell sample from each well were transferred to a 96-well assay plate. Using our iQue® T Cell Activation Kit, we measured different T cell subpopulations expressing the T cell activation markers CD69, CD25 and HLA-DR, cell proliferation and viability and quantitated the levels of secreted INFγ and TNFα from a single well. Data acquisition was done using an iQue® Advanced Flow Cytometry System and the high-content data was analyzed using the integrated iQue Forecyt® software and iQue® T cell activation assay template. The data show dose and temporal responses to each compound with unique activation signatures. This assay provides high-content T cell activation profiles with rapid assay turnaround time and minimal sample volumes for all applications requiring the analysis of T cell activation.

This study highlights the power of the iQue® Advanced Flow Cytometry Platform to rapidly characterize multiple endpoints and the iQue Forecyt® software to provide high content visualization that reveals actionable insights to drive faster decisions in the development of cell therapies.

Methods

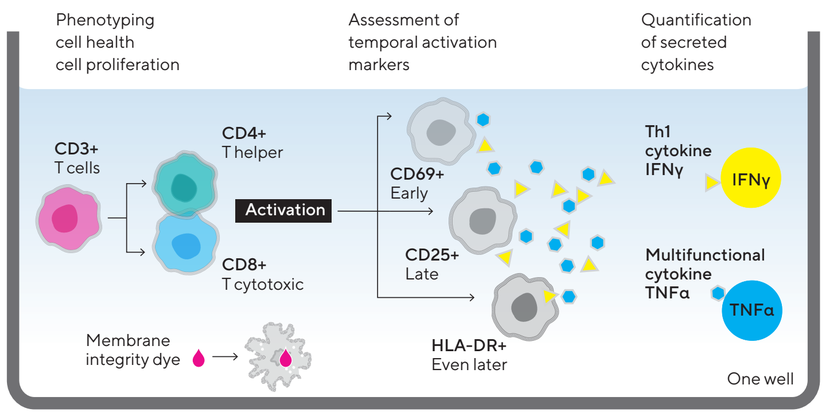
Cryopreserved PBMCs from a single donor were cultured for 24 hours and then stained with the Cell Proliferation and Encoder Dye B | Green (iQue® T Cell Activation Kit, Sartorius). Cells were stimulated with 3 different T cell activators (CD3 | 28 Dynabeads, phytohemagglutinin (PHA), or Staphylococcal enterotoxin B (SEB)) using a 12-point, 2-fold serial dilution series. On days 1, 3 and 6 after stimulation, 10 mL of sample containing cells and supernatant were transferred to an assay plate and analyzed using the iQue® T Cell Activation Kit. Data was acquired on the iQue® system in approximately 15 minutes per 96-well plate. Event gates and all experimental metrics including cell phenotype, T cell activation markers, cell proliferation and viability, and quantitation of secreted cytokines were auto generated using the integrated iQue Forecyt® software.

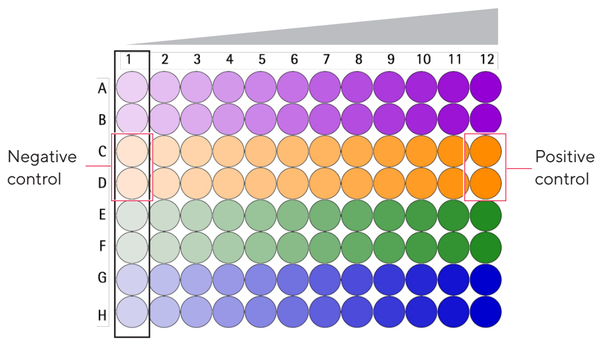
The iQue® Can be Used in Multiple Steps in the CAR-T Bioprocessing Workflow



	T Cell ID			Cell Surface Activation Markers			Secreted Effector Cytokines	
	CD3	CD4	CD8	CD69 (early)	CD 25 (late)	HLA-DR (even later)	TNFα	IFNγ
T cytotoxic cells	+	-	+	+|-	+|-	+|-	+	+|-
T helper cells	+	+	-	+|-	+|-	+|-	+|-	+ (Th1)

Assay Biochemistry and Typical Plate Design

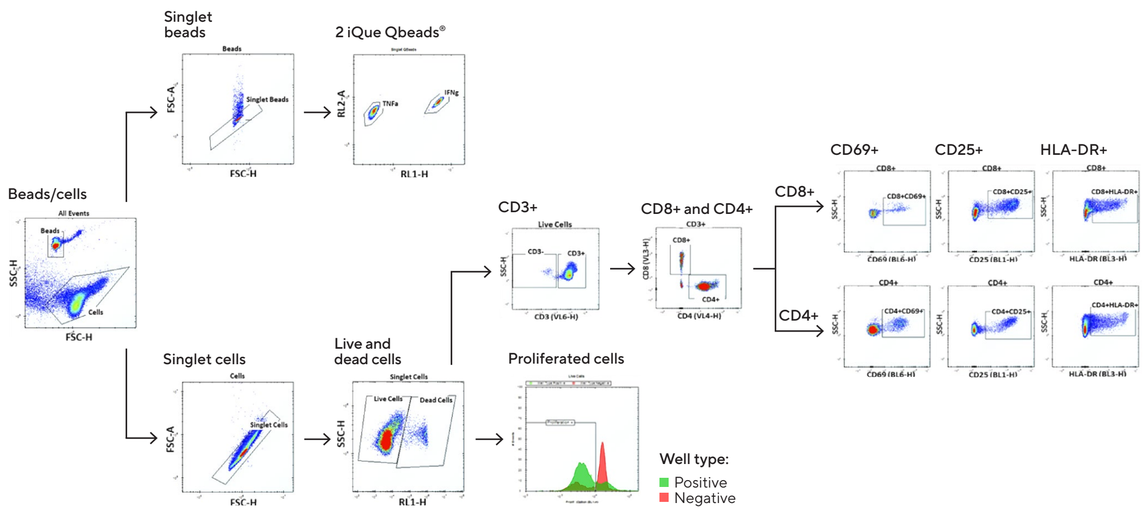




- **INFγ | TNFα Cytokine Standards**
  12-point 1:3 serial titration
  Top concentration: 50,000 pg/mL per Cytokine
- **CD3 | CD28 Dynabeads®**
  12 point 1:2 serial titration
  Top concentration: 1 million/mL
- **PHA**
  12 point 1:2 serial titration
  Top concentration: 10 µg/mL
- **SEB**
  12 point 1:2 serial titration
  Top concentration: 100 ng/mL

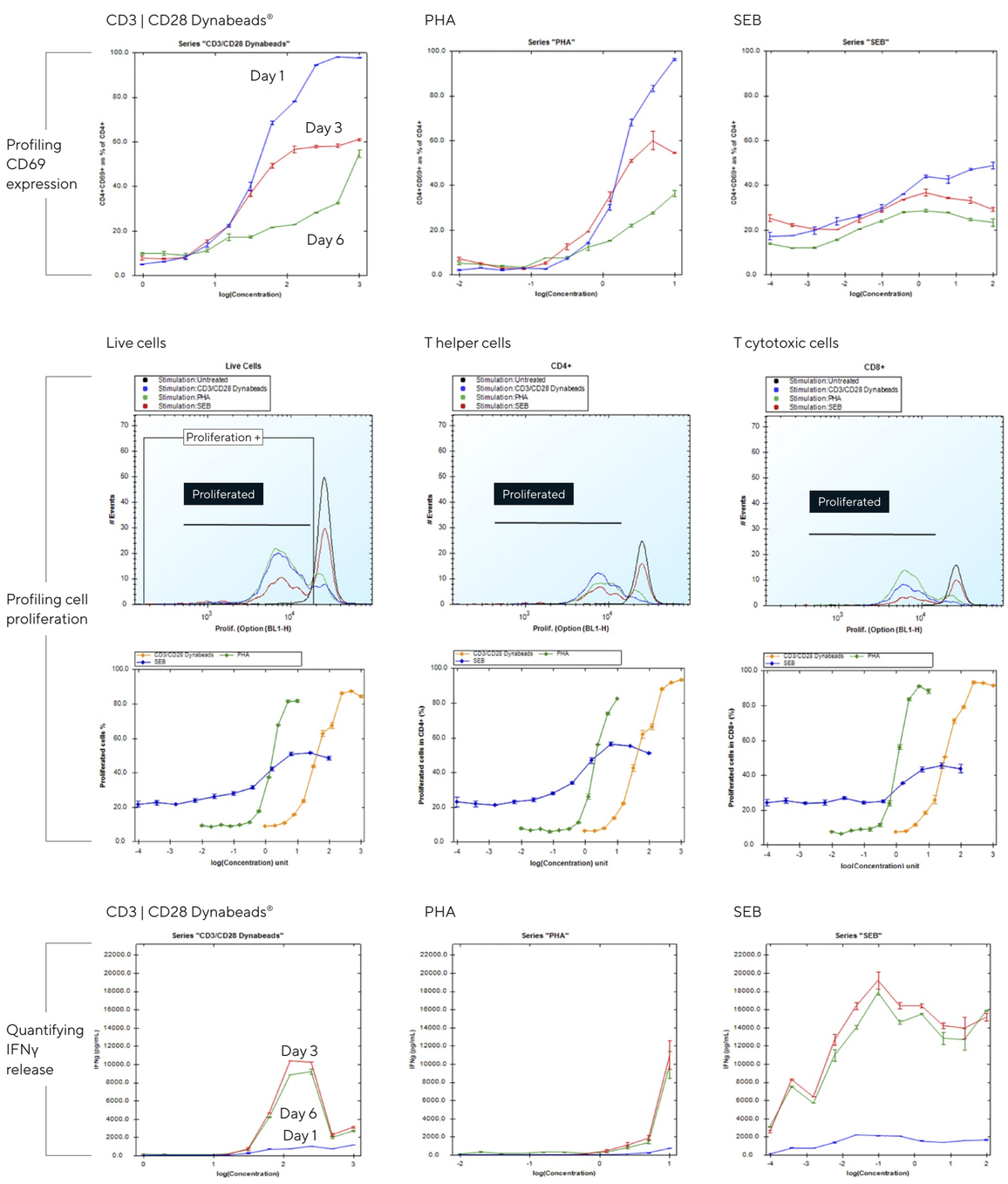
The assay discriminates between live and dead cells by using a membrane integrity dye which only stains dead cells. Live cells in each well are phenotyped by CD3, CD4 and CD8 antibodies to identify the various T cell subsets. Cell surface activation markers are measured to determine early activation (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.

Assay Gating Strategy



The gates and gating strategy are provided by the kit template in the iQue Forecyt® software for ease of use. Briefly, the cytokine capture beads are separated from cells based on differences in size and granularity. The single bead population is resolved into their individual components (IFNγ and TNFα) by their unique fluorescent signatures. Live single cells are detected and then the various T cell subsets are identified. The percentage of each T cell subtype expressing the various activation markers are determined. Cells that proliferated during the experimental time course are identified by a decrease in fluorescent intensity.

Representative T Cell Activation Data



PBMC's were stained with proliferation dye to monitor cell division and were stimulated with 3 different T cell activators. On days 1, 3 and 6 after stimulation, 10 mL of samples were transferred to an assay plate and analyzed using the iQue® T Cell Activation Kit. Data was acquired on the iQue® Advanced Flow Cytometry Platform and the data was analyzed using the integrated iQue Forecyt® software. The data 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 compound.

Summary

iQue® Advanced Flow Cytometry Platform and iQue® T Cell Activation Kit Assay value proposition:

- Multiplex cell and secreted cytokine measurement in a single assay, which disrupts common immunology research workflow that generally requires multiple assays.
- Single platform and data analysis package: streamline data acquisition and analysis workflow and solves the data synchronization issues. No additional color compensation.
- Spatial-temporal analysis of T cell activation at different stages in a single high-content miniaturized assay, saves precious samples and enhances data integrity.
- Optimized assay design that avoids the "guessing" of sample dilution factors and achieves better data integrity.
- Flexible to choose additional cytokines from iQue® T Cell Companion kits for multiplexed analysis. Additional cytokines include: IL-2, IL6, IL-10, IL-13, IL-17A, and GM-CSF.