SARTORIUS

Case Study

Trust in Your Results—Experience the Benefits of iQue® Advanced Flow Cytometry

Case Profile:
To address the demands of modern drug discovery and development workflows, the iQue® platform is built on four key pillars of experimental design:

- Reproducibility
- Reliability
- Optimized sample usage
- Multiplexing

This case study explores those four pillars, demonstrating the benefits of a comprehensive solution to accelerating cell-based assay workflows.

Keywords:
iQue® Advanced Flow Cytometry Platform, Post Transcriptional Changes, Drug Discovery and Development, iQue Forecyt® Software, Cell-Specific Parameters, Phenotypic Changes, Reproducibility, Reliability, Optimized Sample Usage

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Flow cytometry is a powerful tool for analysis of cells and cell-based assays in basic research and for drug discovery and development. The applications of this technology are many and varied—ranging from simple cell counts and viability assays to complex intracellular staining—but it is the ability to analyze large numbers of post transcriptional changes that have made it indispensable for modern cell biology labs. Despite first appearing in the 1960s, and the countless advances since then, flow cytometry still has a reputation of being a difficult technique requiring dedicated experts with in-depth knowledge to perform successfully. Sartorius' iQue® advanced flow cytometry platform has been developed to overcome this, putting this powerful technique back into the reach of every lab.

The iQue® platform offers a novel screening solution that streamlines the process from experimental setup and acquisition to analysis and results. Designed specifically with drug discovery and development in mind, it requires minimal assay optimization thanks to an array of standardized protocols and reagents, and the system's dedicated iQue Forecyt® software generates data such as IC_{50}/EC_{50} curves, heatmaps, and graph plot analyses in real time.

Rapid, accurate data generation is critical to project progression, so the iQue® was designed to provide greater biological insights from fewer experiments using less material. It can simultaneously measure cell-specific parameters—such as phenotypic changes and proliferation rates—and quantify secreted factors in a single assay, using volumes as low as 5 µL per well.

Reproducibility

The iQue® has been engineered to produce robust data across replicates, showing good intra- and inter-day correlation between assay plates (Figure 1A and 1B). Wells containing a known quantity of reagent were used to test reproducibility over different plate replicates and days of testing. Four mouse antibody isotypes, IgG1, IgG2a, IgG2b and IgG3 were mixed in a wide range of concentrations and measured across 24 replicate wells. Three plates were run on the same day (Figure 1A) or a single plate was measured on separate days (Figure 1B) using the iQue® Mouse IgG Type and Titer Assay Kit. Variability was analyzed across all samples, achieving a correlation $R^2 > 0.99$, with linear regression curve values of ~ 1, showing outstanding assay reproducibility for both intra- and inter-day testing.

Figure 1: *Intra- and Inter-Day Reproducibility*

A. Intra-Day Single Point Testing

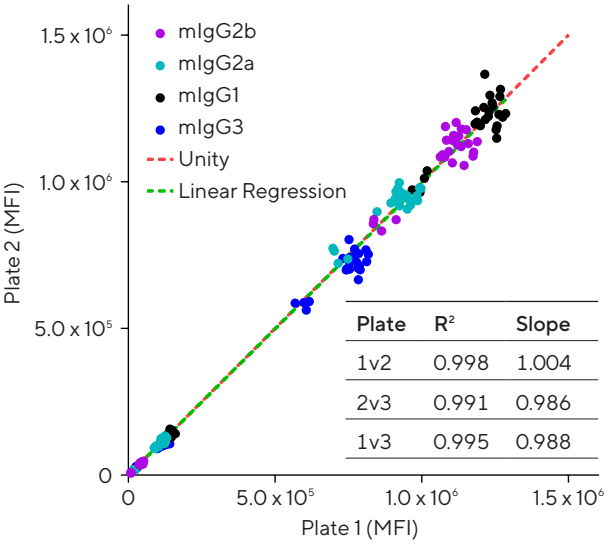
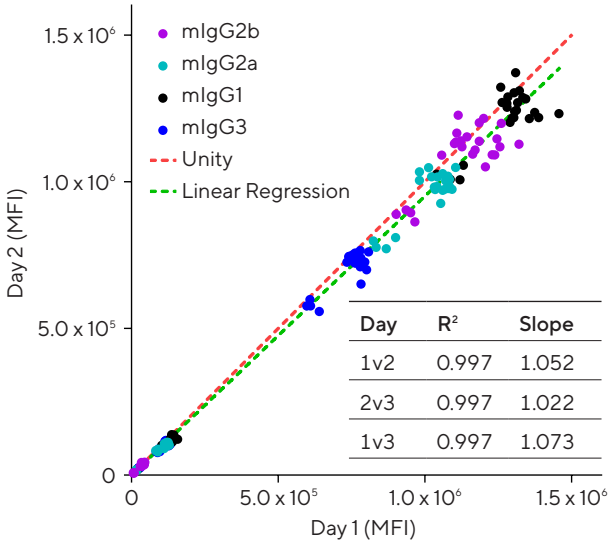


Plate	R^2	Slope
1v2	0.998	1.004
2v3	0.991	0.986
1v3	0.995	0.988

B. Inter-Day Single Point Testing



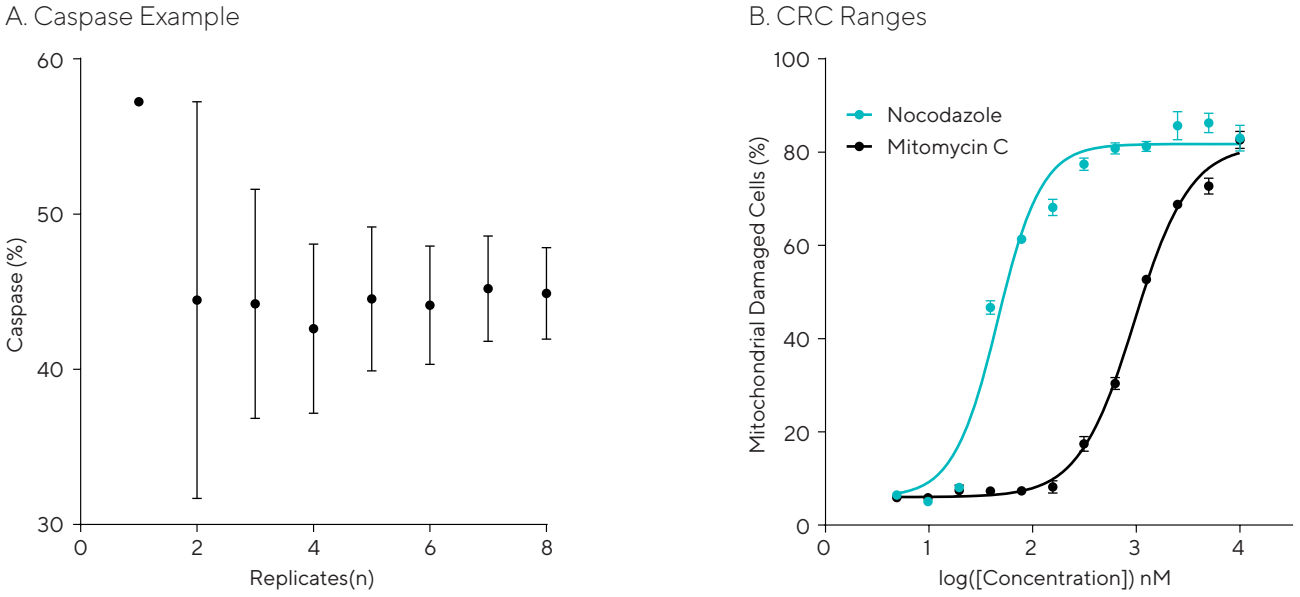
Day	R^2	Slope
1v2	0.997	1.052
2v3	0.997	1.022
1v3	0.997	1.073

Note. iQue® output data showcasing the reproducibility of repeated measurements over different assay plates (A) or days (B). (MFI, mean fluorescence intensity)

The iQue Forecyt® software has been designed to further accelerate workflows by generating concentration response curves and monitoring pharmacological parameters—such as EC_{50}/IC_{50}—without the need for manual data extrapolation. This is crucial for early phase drug discovery, where there is often a trade-off between robustness of data, i.e., the number of replicates performed, and resource use in terms of user time, sample availability, and reagent costs. Automating sample processing and analysis, combined with assay miniaturization, enables researchers to create robust and reproducible data sets faster and more cost-effectively. To demonstrate this, a camptothecin-induced apoptosis assay was performed in MDA-MB-231 cells, exemplifying how confidence in data increases with a higher number of sample replicates (Figure 2A). Eight replicates of 10,000 cells per well were plated out, and apoptosis was induced over 72 h. The percentage of caspase-positive cells was measured, and the standard error of the mean (SEM) calculated, from a single sample to all eight replicates. As expected, a higher number of replicates yielded lower SEM for more robust data.

This data analysis functionality, combined with iQue®'s broad dynamic range, makes it possible to test more concentrations in less time, allowing experiments to span a wide range of concentrations in a single plot. For example, concentration response curves can be generated for two compounds with very different potencies—nocodazole (EC_{50} = 45.9 nM) and mitomycin C (EC_{50} = 960.7 nM)—using a single, broad concentration range. This is shown in Figure 2B, where Jurkat cells were plated at $1x10^6$/mL and incubated for 24 h with either nocodazole or mitomycin C, using a mitochondrial damage readout to measure the induction of apoptosis.

Figure 2: *Reproducibility Across Replicates and Wide Concentration Ranges*

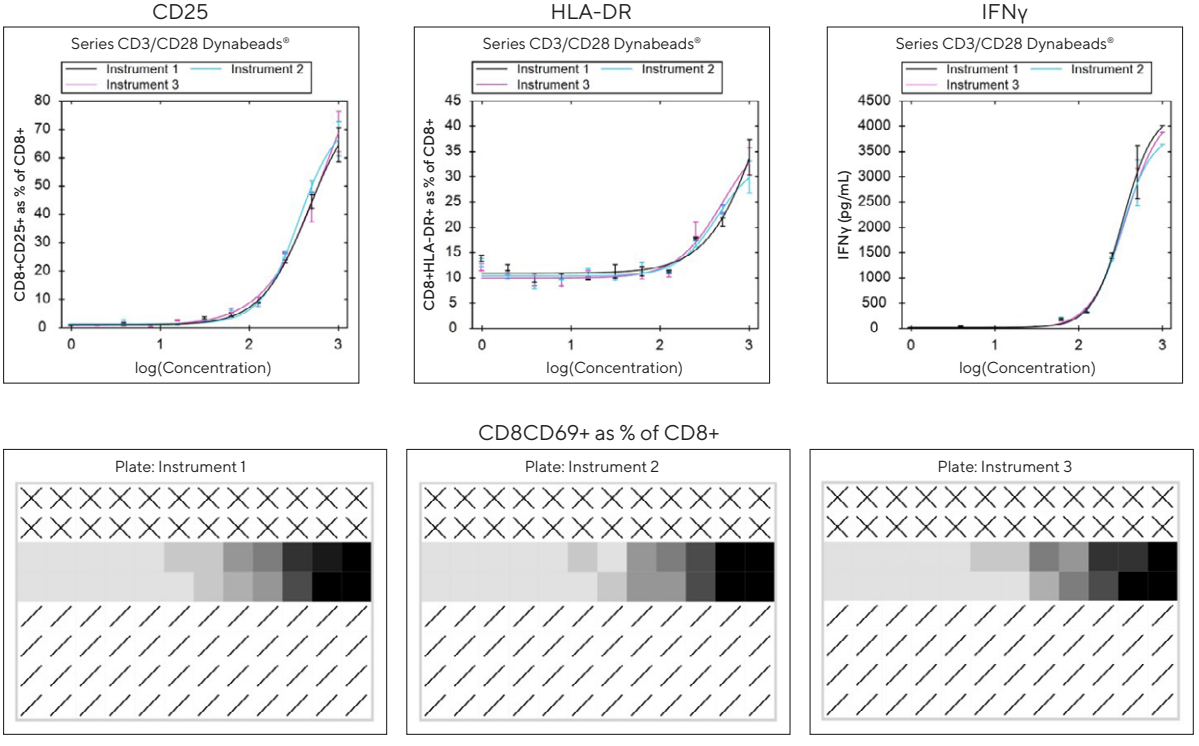


Note. (A) Example of decreased assay variability with number of replicates. (B) Software analysis of concentration responses include a broad range of different potency compounds.

Reliability

The iQue® is designed to provide users with consistent data across different individual instruments or operators, offering dependable operation and remarkable consistency between runs using a fixed photomultiplier tube (PMT) voltage. To showcase this accuracy, peripheral blood mononuclear cells (PBMCs) were stimulated with CD3/CD28 Dynabeads® for 24 hours, and immunophenotyped for expression of T cell activation markers and soluble cytokines with the iQue® Human T Cell Activation Kit. Concentration response curves (Figure 3, top) or heat map analyses (Figure 3, bottom) support the reliability of data collected across three separate instruments, and from assays performed by different operators.

Figure 3: *Data Reliability*



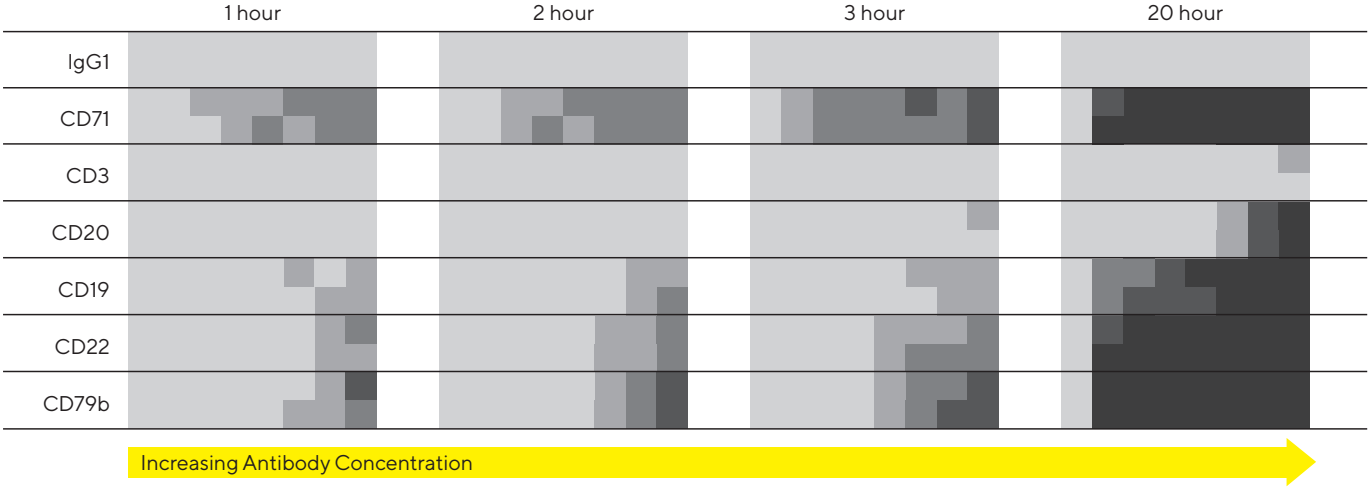
Note. Overlapping experimental results achieved on different iQue® instruments and with different operators [IFNγ, interferon gamma].

Optimized Sample Usage

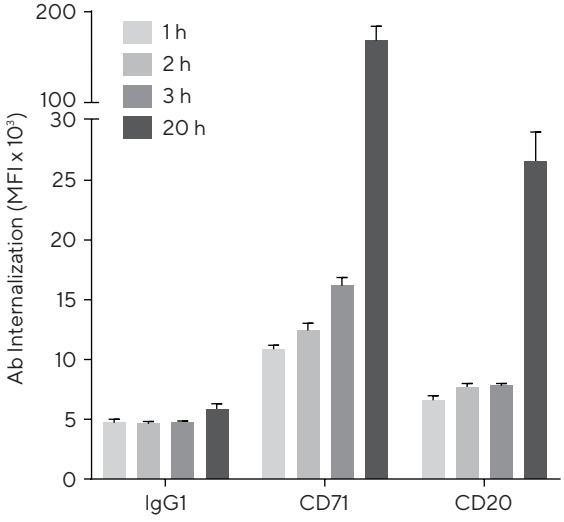
The sequential sampling and miniaturization capabilities of the iQue® allow multiple parameters to be evaluated from a single well over time, conserving valuable samples and reagents, and reducing experimental variability. To demonstrate this, the iQue® Multiplex Human Antibody Internalization Kit—an antibody internalization assay—was run for 20 hours with repeated evaluation of multiple parameters, including cell viability. Ramos cells were plated at $1x10^6$ cells/mL in 384-well plates containing a two-fold serial dilution of antibodies—starting at 1 mg/mL—and sequential measurements were performed for each well at various time points. Heatmaps (Figure 4A) and graphs (Figure 4B) present data analysis for the degree of antibody internalization at every sampled time point, demonstrating the importance of evaluating multiple time points for detection of antibodies with slower internalization rates, as exemplified here by CD20.

Figure 4: *Antibody Internalization Time Course*

A. Antibody Internalization: Ramos Cells



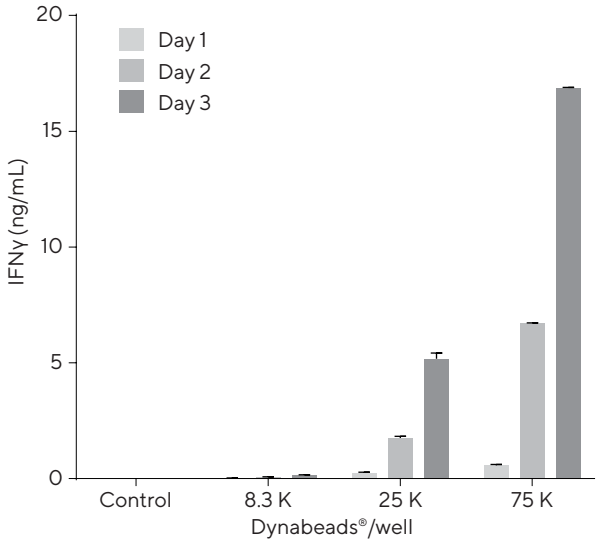
B. Antibody Internalization Using 1 mg/mL



Note. The iQue® allows multiple measurements of different variables from as little as 5 µL aliquots over an experimental time course. (MFI, mean fluorescence intensity; Ab, antibody)

Repeated sampling with the iQue® is also ideal for tracking dynamic biological changes over time, such as quantification of cytokine secretion following activation of cells. To demonstrate this, a sequential three-day interferon gamma (IFNγ) release assay was performed after co-culturing Ramos cells and PBMCs at a 5:1 ratio in the presence of a range of CD3/CD28 Dynabeads® concentrations (Figure 5).

Figure 5: *Measuring Biological Factors over Time*

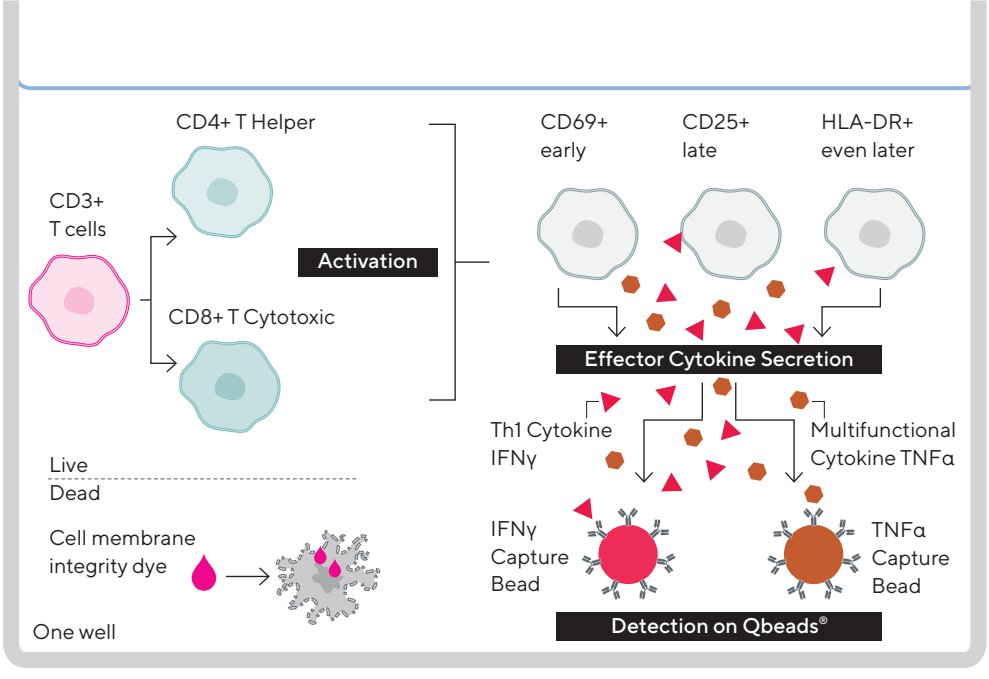


Note. The iQue® can measure biological changes, such as IFNγ release in an immune cell killing (ICK) assay, over a defined experimental time course from a single well.

Multiplexing

The last pillar, and yet of increasing demand among drug discovery labs, is the iQue®'s ability to perform multiparametric measurements to generate more biologically-relevant data. With validated, ready-to-use multiplexed assay kits, the iQue® is ideally placed to handle several plates and provide multiple readouts, using no wash | single wash assays and novel data reduction tools to deliver accurate and precise results while relieving bottlenecks in the laboratory workflow. The iQue® can simultaneously measure changes in cell phenotype, proliferation, and apoptosis—in combination with secreted protein release (e.g., cytokines), bringing a new meaning to high-content analyses. To demonstrate this, a single well T cell assay was performed to track both cell surface activation markers—CD25, CD69 and the antigen presenting molecule HLA-DR (as a measure of late-stage activation)—and activation-induced secreted factors in the cell media (Figure 6).

Figure 6: *Multiplex Assay Set-Up*

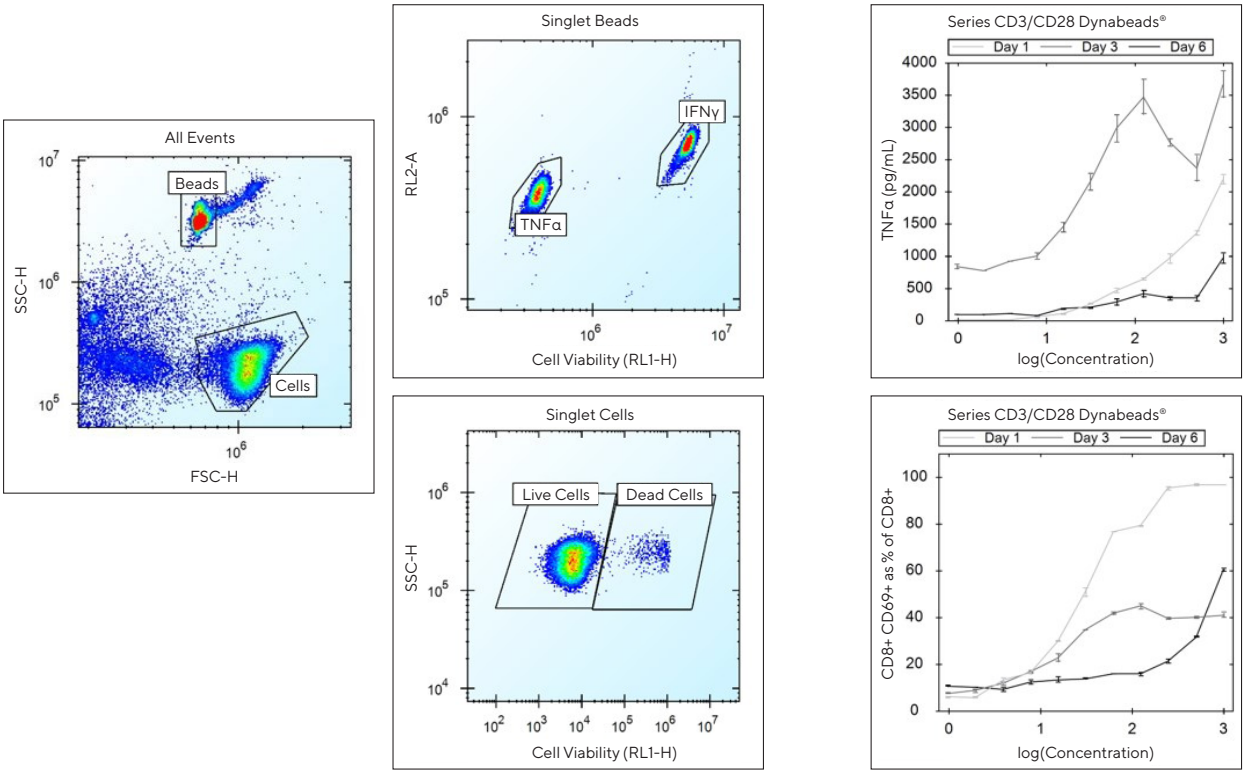


Note. T cell activation is tracked over a defined time course by simultaneously monitoring cell surface markers and secreted cytokines.

iQue®'s validated protocols and predefined analytic templates make these assays easy to set up and run, with optimized workflows that significantly decrease operator time and allow quick transition from data acquisition to analysis, including plate level analytics, dynamic profiling of multiparameter data sets and easy visualization. To exemplify the iQue®'s potential as a multiparameter analyzer, a six-day T cell assay was performed. PBMCs were initially stimulated with varying concentrations of CD3/CD28 Dynabeads®, and the iQue® Human T cell Activation Kit was used for synchronized multiplexed measurements—viability, phenotype, surface markers and secreted proteins—of each sample. Gating strategies (Figure 7A) and time course measurements (Figure 7B and 7C) are easily visualized in real time, without the need for data to be exported and analyzed using different software packages. This approach combines flow cytometry assays and ELISAs, and data from both is collected from a small volume of sample, without the need for multiple plates per assay or time point.

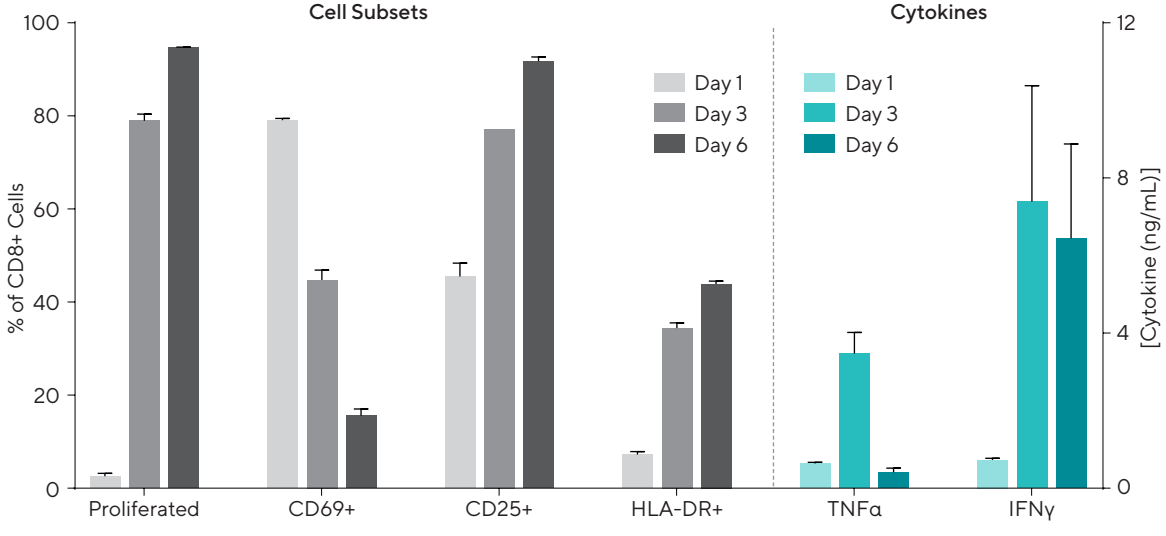
Figure 7: *Single-Well, Multiparametric Readouts*

A. Gating Strategy



B. Time Course

C. Single Bead Concentration Multiple Parameters Over Time



Note. Multiple readouts are collected from a small volume and a single well, exemplified by a T cell activation assay, where measurements were collected from beads and cells simultaneously [TNFα, tumor necrosis factor alpha].

Summary

The examples presented in this case study demonstrate the suitability of the iQue® as a reliable platform for high-content, high-throughput cellular analyses. The system has been developed with drug discovery and development labs in mind, where quick, reproducible, and multiplexed screening platforms are in increasing demand. Combined with iQue Forecyt® software, which provides a fast transition from acquisition to analysis within minutes, data can be easily visualized and quickly interpreted without the need for data extrapolation and export, even for complex biological assays. Easy to set up, reliable and robust, with low sample volume requirements to allow repeated measurements from a single well, the iQue® brings the flow back into flow cytometry.

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