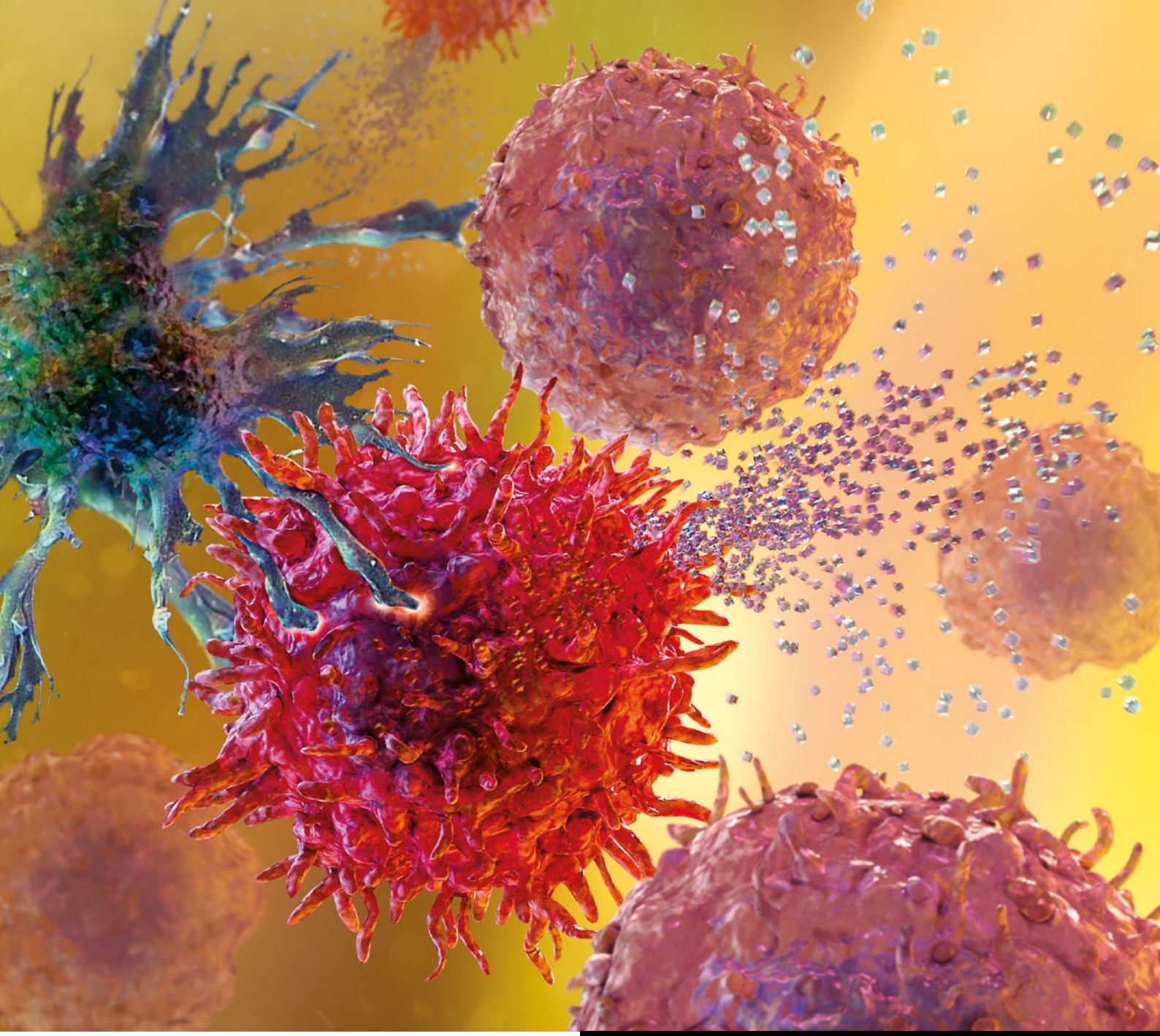


iQue® Reagent Kits

High-Throughput, Multiplexed Solutions for Faster Time to Actionable Answers

Simplifying Progress

SARTORIUS

iQue® Reagent Kits

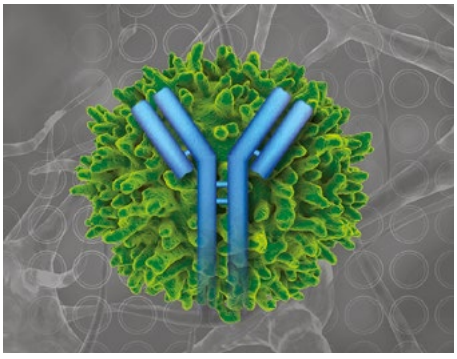
iQue® Reagent Kits provide an integrated solution to enable high content, high-throughput cytometry based analysis for insight into complex biology. iQue® Reagent Kits have been developed to be run on the iQue® high-throughput screening (HTS) cytometry platforms and analyzed with iQue Forecyt® software.

- Biological insight: Multiparametric data gives a fuller understanding of cell populations for more informed actionable results.
- Speed: Analyze a 96-well plate in as few as 5 minutes or a 384-well plate in as few as 20 minutes for a faster time to result.
- Ease of use: A streamlined workflow utilizing single platform assay and data analysis with iQue Forecyt® eliminates the time required for acquiring and correlating data from multiple sources.
- Miniaturize assay volumes: Use as little as 5 µL, conserving precious samples and reagents for additional analysis.

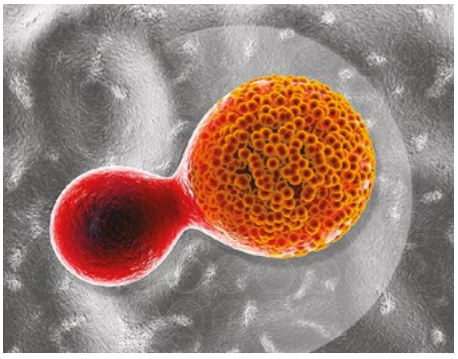
iQue Qbeads® and iQue® Qpanels Kits allow you to capture and analyze specific proteins on distinct bead types for multiplexed quantitation of secreted cytokines, adhesion molecules, enzymes, growth factor receptors, and more.

iQue® Reagent Kits enable the measurement of multiple functional readouts, including cell cycle, apoptosis, membrane integrity, proliferation, and antibody characterization, as well as a variety of immune cell functions on beads, cells, or both, allowing you to simultaneously assess both phenotype and function.

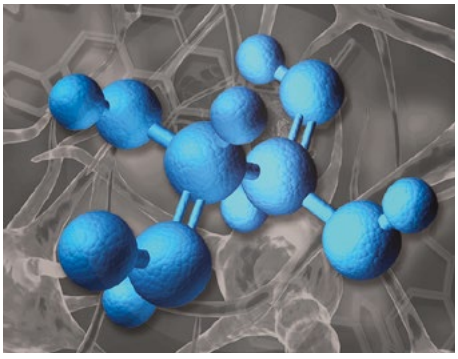
iQue® Reagent Kits



Antibody Discovery and Development
Increase data throughput and quality by multiplexing antibody binding, function, and titer across the process.
- Antibody screening
- Functional profiling
- Cell line development



Adoptive Cell Therapy
Assess multiple cell parameters faster, with fewer cells and less reagents.
- Immune cell killing
- Immune cell assessment
- Cytokine profiling



Small Molecule Screening
Perform high-content phenotypic screening of immune biology across the drug discovery process.
- Primary immune cell screening
- Immune function
- Mechanism of action

Kits at a Glance

Kit Type	Key Advantages	Suitable Applications	Page
iQue Qbeads® Plexscreen Kits [2,3] Capture human, mouse, or rat secreted proteins	Configurable to meet your needs—plex up to 30 analytes from our menu and use pre-configured analysis templates	Cytokine profiling, drug screening, and biomarker discovery	4
iQue Qbeads® Devscreen Kits [3] Attach your own target proteins or capture antibodies for greatest flexibility	Plex up to 30 of your own analytes with iQue Qbeads® Devscreen SH, or 5 analytes with iQue Qbeads® Devscreen SAv	Cytokine profiling, phenotypic screening, and antibody screening	4
iQue® Immune Cell and Bead-Based Kits [1] Assess immune phenotype and function in a single assay	T Cell Characterization and Immune Cell Killing Kits measure immunophenotyping markers, cell health, cell function, and cytokine profiling markers in a single well Highly reproducible, streamlined workflow with minimal hands-on time	Cytokine profiling, T cell biology, immunophenotyping, immune cell killing, and characterization	6–11
iQue® Cell and Bead-Based Kits for Antibody Characterization [1,2] Screen clones and speed up antibody discovery efforts High throughput evaluation of antibody internalization	Analyze both cells and secreted proteins simultaneously in a single assay Generate data for informed decisions from less sample, faster than conventional assays	Clone selection, IgG titer, isotyping, cell health, and antibody internalization	
iQue® Cell-Based Kits [1,2,3] Understand cell cycle, cell health, apoptosis, membrane integrity, and proliferation Encode multiple cell lines for multiplexed analysis in a single well	Measure multiple endpoints of cell health and cell function across a breadth of biological processes like cell activation, differentiation, communication, and death	Cell cycle, apoptosis, cell membrane activity, membrane target antibody screening and proliferation status	

1. Available in both 96-well and 384-well formats
2. No-wash protocol for improved reproducibility and reduced assay time
3. Flexibility to multiplex with other kits

Cytokine Profiling and Bead-Based Screening
iQue Qbeads®

iQue Qbeads® are a family of reagents that enable the capture of specific proteins on distinct bead types, enabling the multiplexed quantitation of biological parameters such as secreted cytokines, adhesion molecules, enzymes and growth factor receptors using minimal sample volume and a simple, fast workflow. iQue Qbeads® come in two variations: iQue Qbeads® Plexscreen Reagents and iQue Qbeads® Devscreen Kits.

iQue Qbeads® Plexscreen Kits

- Choose from over 50 analytes to quantify human, mouse or rat secreted proteins.
- Configure your own panel for analysis of up to 30 secreted proteins in a single well using our online assay-builder tool.

Please visit our website to build a panel and request a quotation

- Combine with other iQue® kits, with no reduction in analysis speed.
- Simplified no-wash and one-wash protocols.
- Kits include detection reagents, standard protein, buffers and pre-defined analysis templates (Figure 1), providing the fastest sample to decision workflow.
- Quantitative readouts measured as fluorescence intensity, or interpolated to a concentration (pg/mL) in solution via the use of a standard curve (Figure 2).

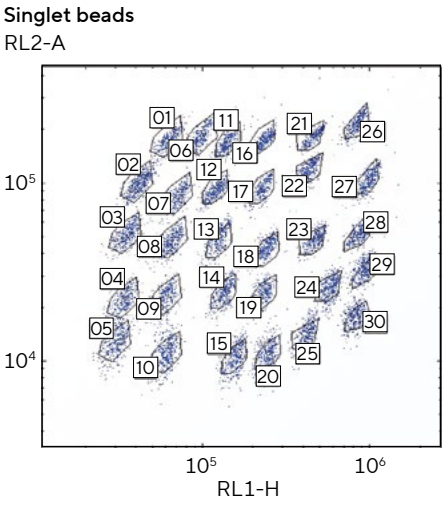


Figure 1: Pre-defined analysis templates are provided with each kit for the included analytes. Bead population gates are empty before acquisition, and are populated with the appropriate beads during sample acquisition.

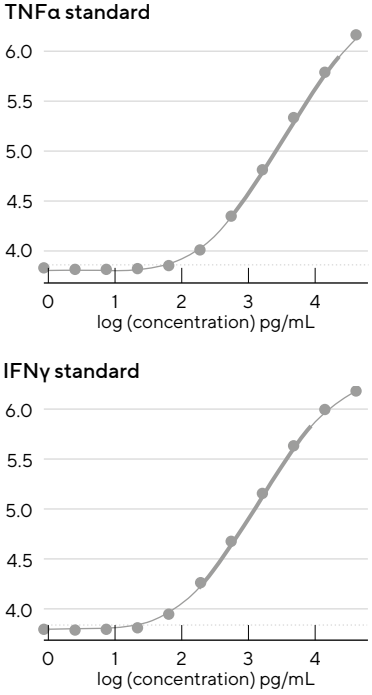


Figure 2: Sample standard curves generated for different cytokines.

iQue Qbeads® Devscreen Kits

iQue Qbeads® Devscreen kits allow users the flexibility to attach their own capture antibodies or target proteins onto iQue Qbeads®. Devscreen beads come coated with either Streptavidin or Sulfhydryl functional groups.

iQue Qbeads® Devscreen Streptavidin Coated Reagents

- Streptavidin coated kits used for screening (Figure 3).
- Available in 5 different bead populations.
- Multiplex (by bead size) with analytes from the Plexscreen or Devscreen Sulfhydryl (SH) panels.

iQue Qbeads® Devscreen Sulfhydryl Derivatized Reagents

Find out more

- Sulfhydryl coated beads covalently bind any molecule with a free amine functional group in a simple two-step process.
- Available in up to 14 different bead populations.
- Multiplex with iQue Qbeads® Plexscreen and Devscreen SAv reagents kits (multiplexing with iQue Qbeads® Plexscreen is subject to bead compatibility).

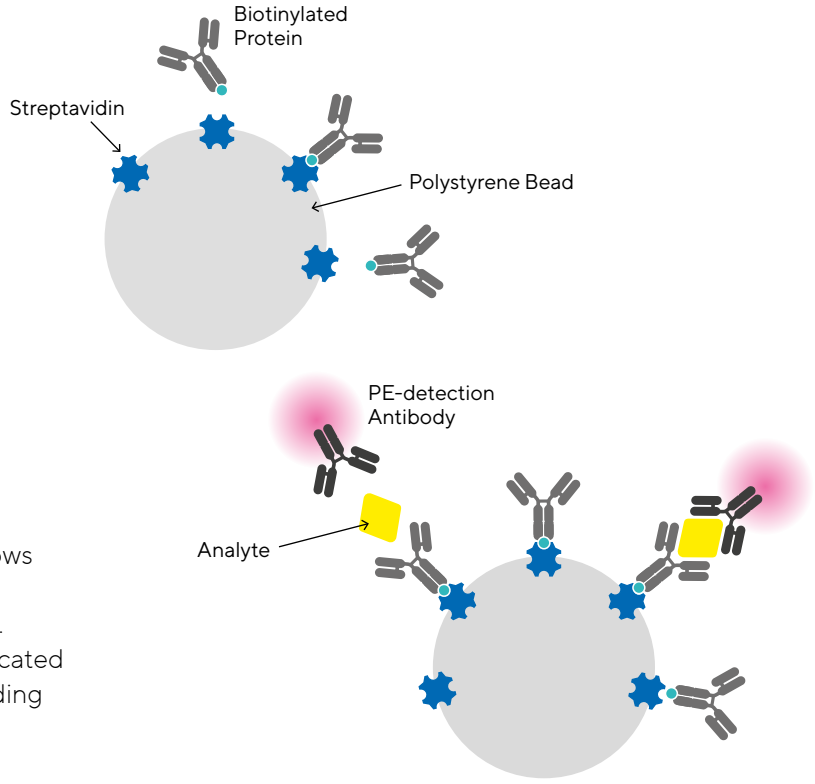


Figure 3: Principle of iQue Qbeads® Devscreen Streptavidin Coated Reagents

iQue Qbeads® Human Inflammation Panel Kit

iQue Qbeads® Human Inflammation Panel Kit allows the measurement of seven human cytokines and chemokines from either serum or *in vitro* samples. The cytokines and chemokines included are implicated in inflammatory responses to disease states including autoimmune diseases, chronic inflammation, and infections. Analytes offered in the iQue Qbeads® Human Inflammation Panel Kit include: Human Interferon gamma (IFNγ), Interleukin-2 (IL-2), Interleukin-6 (IL-6), CCL2 (MCP-1), CCL3 (MIP-1α), CXCL9 (MIG), and CXCL10 (IP-10).

Please visit our website to build a panel and request a quotation

Immune Cell Function and Characterization

iQue® Immune Cell and Bead-Based Kits

iQue® Human T Cell Activation Kit

The iQue® Human T Cell Activation Kit streamlines the traditional workflow by measuring immune cell phenotypes, early and late markers of T cell activation, cell proliferation or encoded target cells, cell viability and secreted cytokine concentrations (IFNγ and TNFα) using only 5 µL–10 µL of samples. (Figure 4).

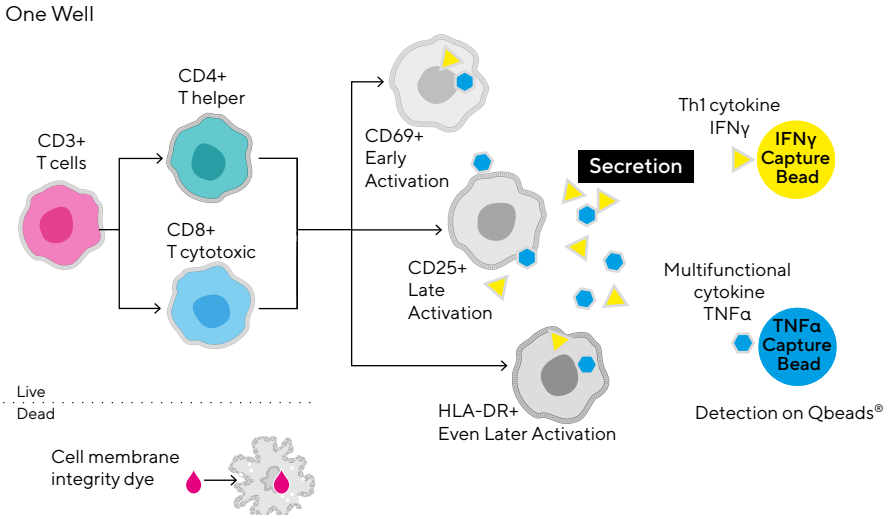


Figure 4: Simultaneous measurement of T cell phenotypes, activation markers, cell proliferation, cell viability, and secreted cytokines in a single well. Measurement of T cell proliferation or encoded target cells is possible, but is not included in this illustration.

iQue® Human T Cell Exhaustion Kit

The iQue® Human T Cell Exhaustion Kit is designed for ease of use in multiplexing markers of T cell exhaustion, phenotyping T helper and T cytotoxic cells, assessing cell health, and bead-based measurement of secreted cytokines, all in the same assay (Figure 5).

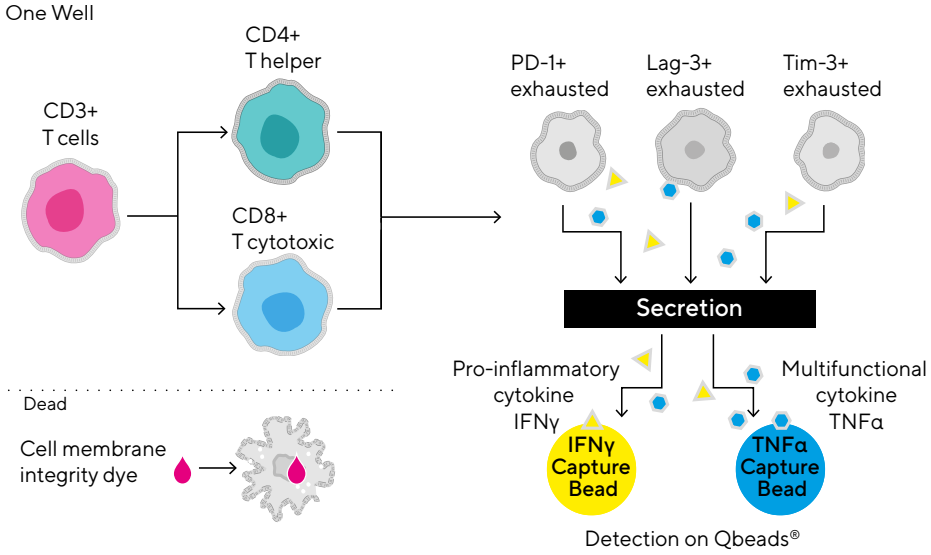


Figure 5: Simultaneous measurement of cell viability, T cell phenotypes, T cell exhaustion markers, secreted cytokines, cell encoding and proliferation (optional) in a single well. Measurement of T cell proliferation or encoded target cells is possible but is not included in this illustration.

Find out more

iQue® Human T Cell Memory Kit

The iQue® Human T Cell Memory Kit measures T cell memory phenotypes and function at different stages while providing information about their health and their role in cytokine secretion. This one-wash assay requires minimal hands-on time and measures both cells and beads together in a single well (Figure 6).

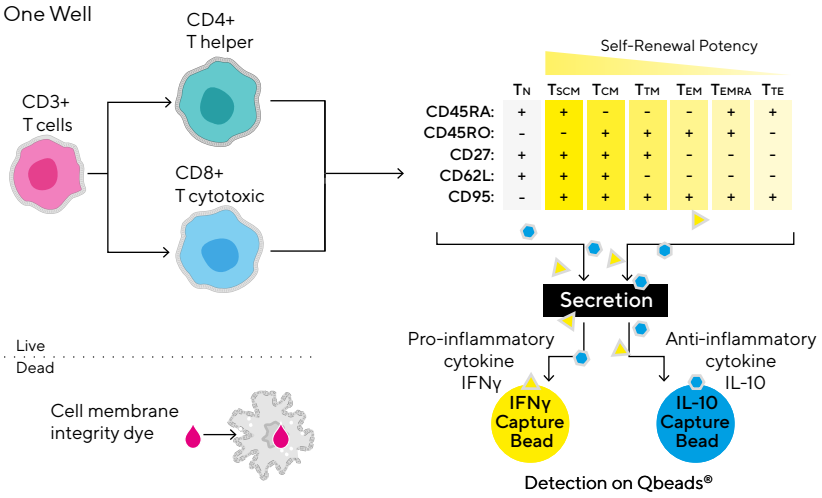


Figure 6: Simultaneous measurement T cell phenotypes, T memory subsets, cell health, secreted cytokines in a single well.

iQue® Human T Cell Companion Kits

iQue® Human T Cell Companion Kits are used in combination with the iQue® Human T Cell Activation Kit and iQue® Human T Cell Memory Kit to allow the measurement of up to six more human cytokines in addition to those already included in the iQue® Human T Cell Activation Kit and iQue® Human T Cell Memory Kit. The iQue® Human T Cell Companion Kits are supplied with their own pre-formatted analysis template (Figure 7).

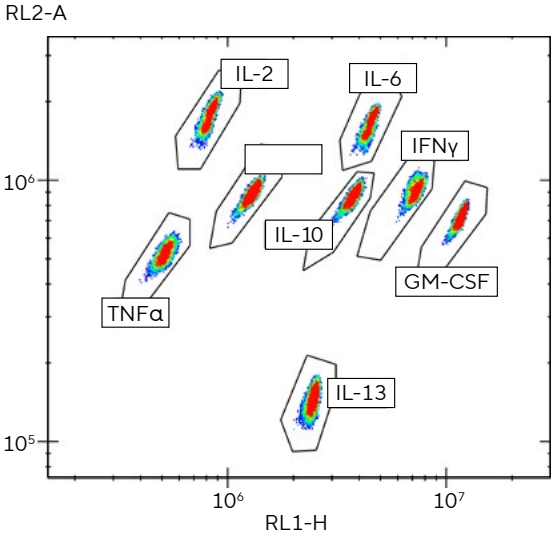


Figure 7: iQue® Human T Cell Companion Kit cytokines template.

Find out more

iQue® Human T Cell Phenotyping Kit (CD3, CD4 and CD8)

The iQue® Human T Cell Phenotyping Kit (CD3, CD4 and CD8) is designed for reliable identification of human T cell subsets (Figure 8). This assay is optimized to run on the iQue® 3 HTS Cytometry Platform configurations, which combine high throughput sampling, flow cytometry detection and multiplexing capabilities. The kit is formulated to minimize non-specific background staining. The optimized workflow also provides the flexibility by enabling additional cytokines to be added for further characterization of subpopulations.

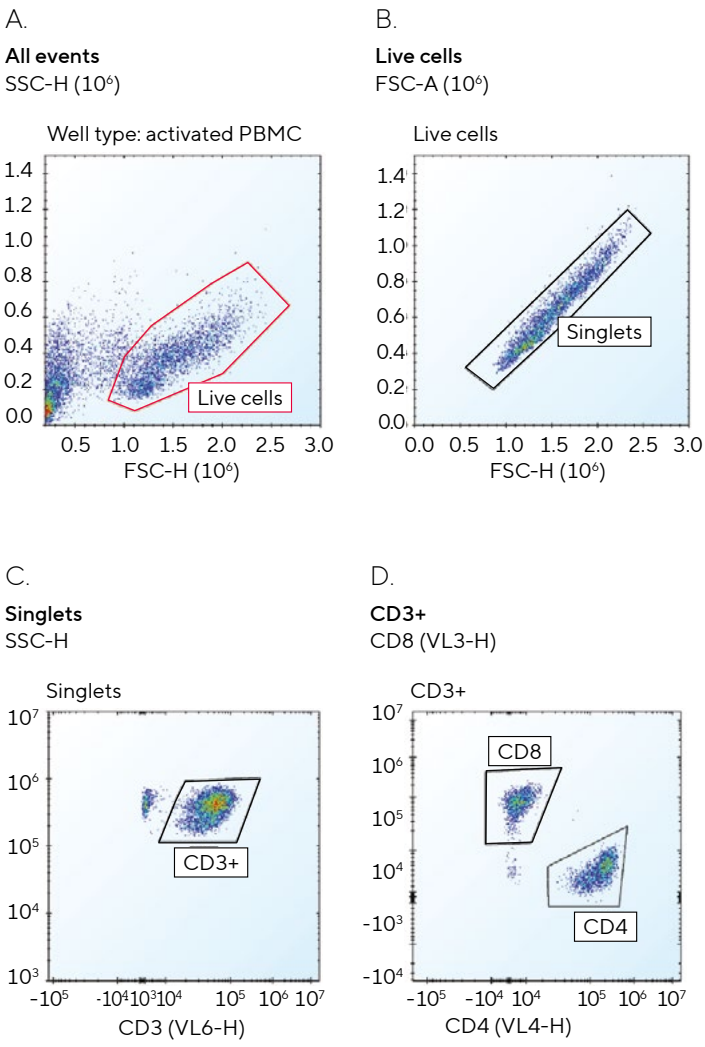


Figure 8: Phenotyping analysis of human PBMCs activated with CD3|CD28 antibodies for 3 days. (A) Set gate for live cells in all events to exclude debris | dead cells. (B) Remove doublets from live cells to obtain single cells. (C) CD3+ cells of Singlets. (D) CD4+ and CD8+ subsets of CD3+ cells.

Find out more

Immune Cell Killing Kits

iQue® Human T Cell Killing Kit

The iQue® Human T Cell Killing Kit was designed for ease of use in multiplexing target cell identification, cell health, cell phenotype and function markers along with bead-based, secreted protein profile measurements in the same assay (Figure 9).

One Well

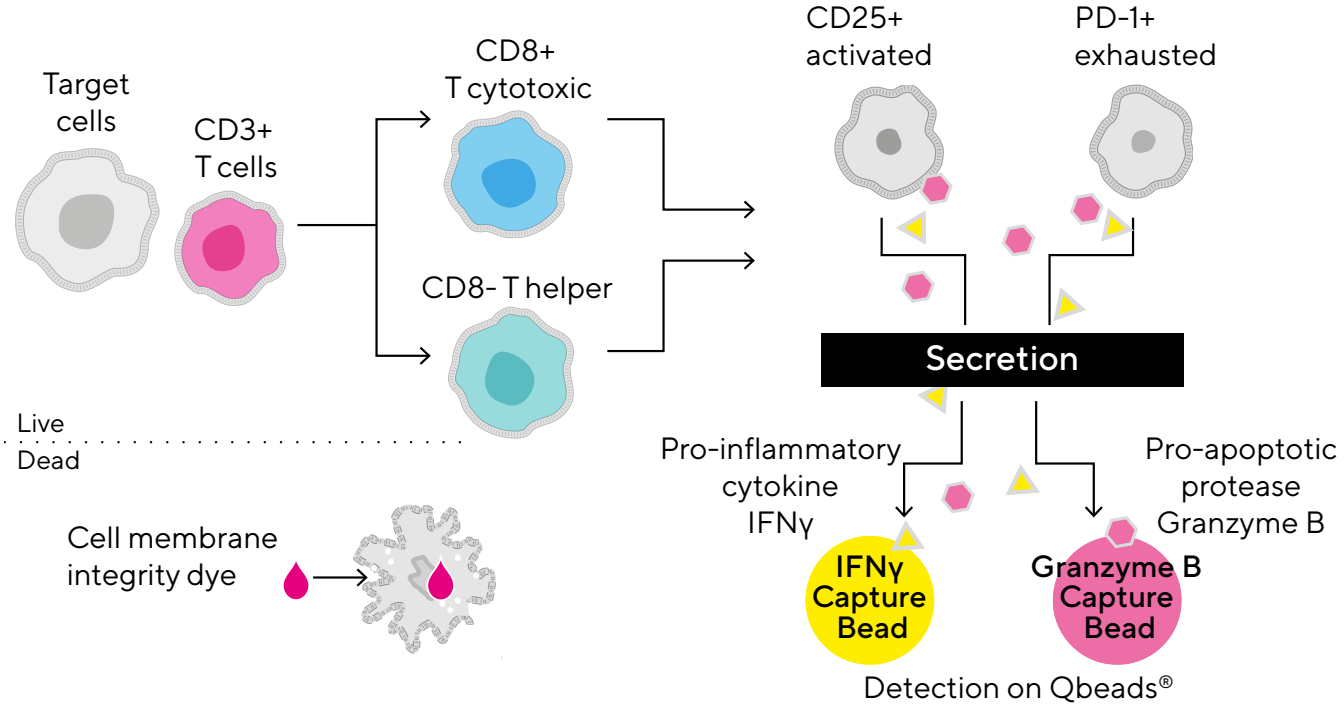


Figure 9: Simultaneous measurement of target cell identification, cell health, cell function, immunophenotyping, and cytokine profiling all in one well. Target cells are distinguished from effector cells by staining with a fluorescent encoding dye (encoding dye is not shown in this illustration).

Find out more

iQue® Human NK Cell Killing Kit

The iQue® Human NK Cell Killing Kit is a cell and bead mixture assay that simultaneously measures cell phenotype markers, NK cell functional markers, target cell identification, effector secreted pro-inflammatory cytokine and pro-apoptotic protease, and lastly cell count and cell membrane integrity (Figure 10).

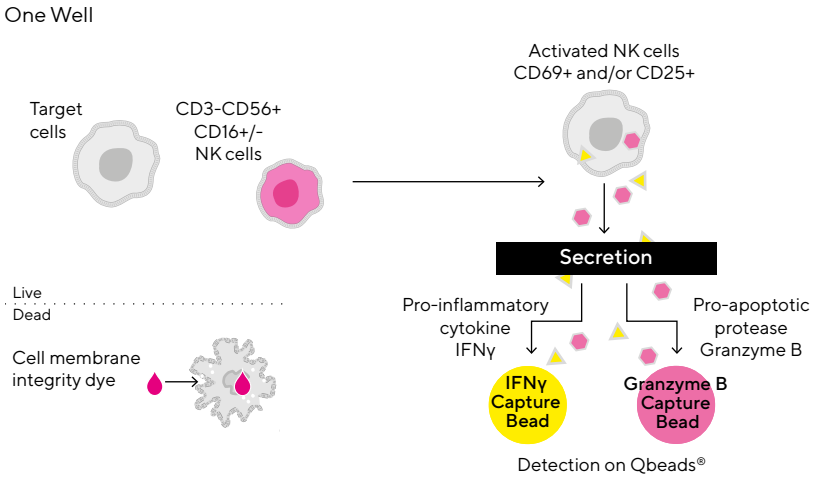


Figure 10: Simultaneous measurement of target cell identification, cell health, cell function, immunophenotyping, and cytokine profiling all in one well. Target cells are distinguished from effector cells by staining with a fluorescent encoding dye (encoding dye is not shown in this illustration).

iQue® Human NK Cell Companion Kits

The iQue® Human NK Cell Companion Kits may be used in combination with the iQue® Human NK Cell Killing Kit to allow measurement of up to 6 additional human cytokines | effector proteins along with IFNγ and Granzyme B which are already included in the iQue® Human NK Cell Killing Kit (Figure 11).

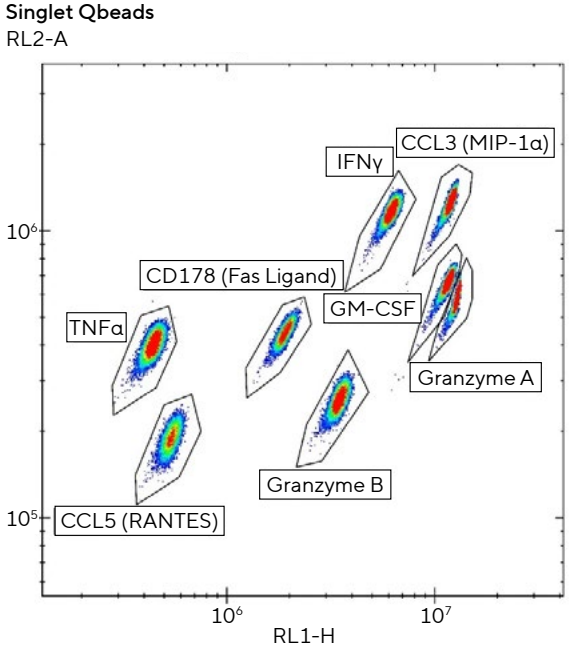
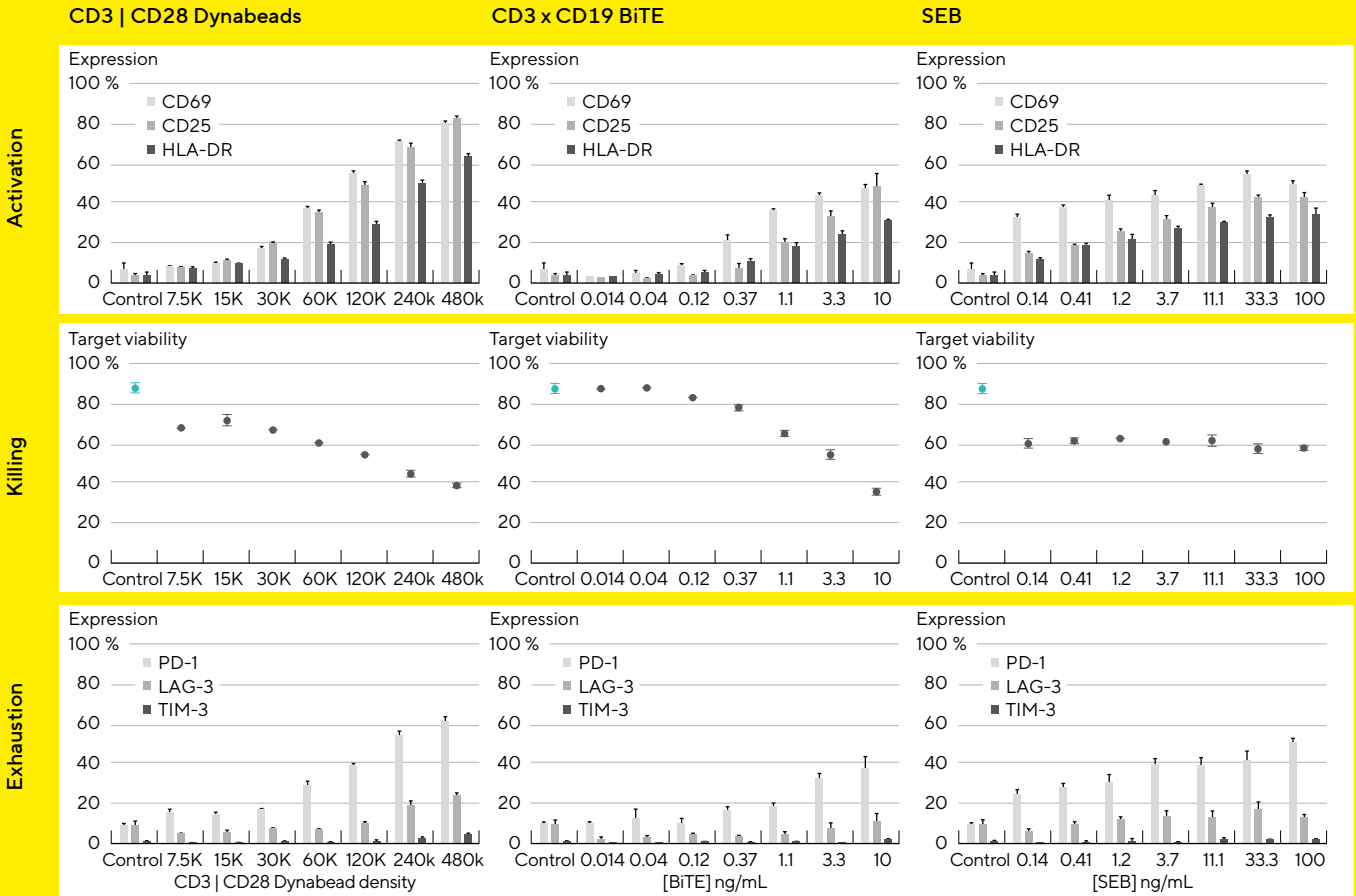


Figure 11: iQue® Human NK Cell Companion Kit cytokine template

Find out more

Application Spotlight: T Cell Characterization

Thorough evaluation of T cell phenotype and function is critical to understanding T cell biology and building better therapeutics. A growing number of immunotherapies, for example bispecific antibodies, checkpoint inhibitors and CAR-T cells, are being developed to target various stages in T cell activation and differentiation pathways. Detailed characterization during the course of T cell development has the potential to offer greater insights leading to improved therapeutics.



Memory

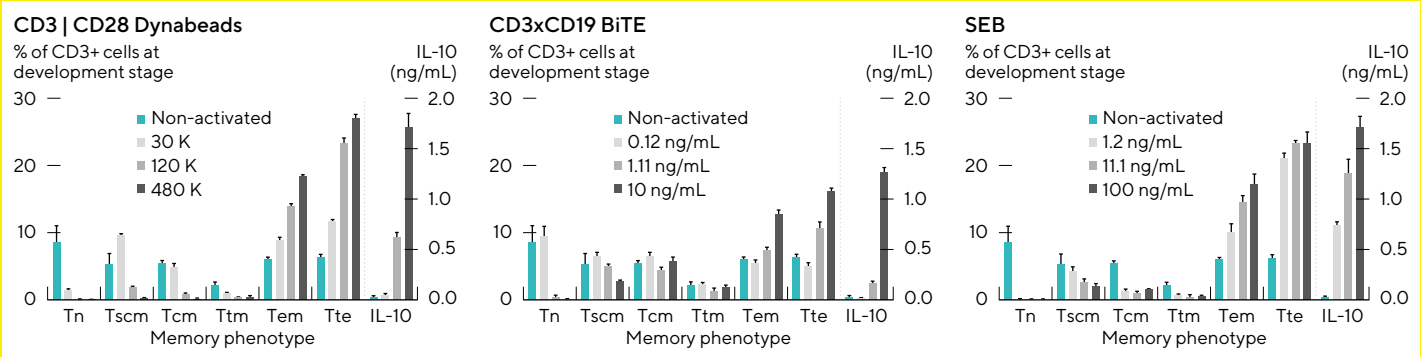


Figure 12: High-throughput cytometry with the iQue® HTS Cytometry Platform provides a robust, high throughput solution for multiplexed studies of T cells. Combined with four of our T cell characterization kits (iQue® Human T Cell Activation Kit, iQue® Human T Cell Killing Kit, iQue® Human T Cell Exhaustion Kit, and iQue® Human T Cell Memory Kit), they collapse the traditional workflow by providing a convenient approach for evaluating cell phenotypes, T cell markers, cell proliferation, cell viability and secreted cytokines, to be measured in a single sample.

Antibody Characterization
iQue® Cell and Bead-Based Kits

iQue® Human Titer and Viability Kit

The iQue® Human Cy-Clone™ Plus enables the rapid analysis of thousands of clones in a simple no-wash, mix and read assay. It is the only solution to correlate IgG quantitation, cell viability and cell count in a single well in order to make more informed decisions on cell productivity.

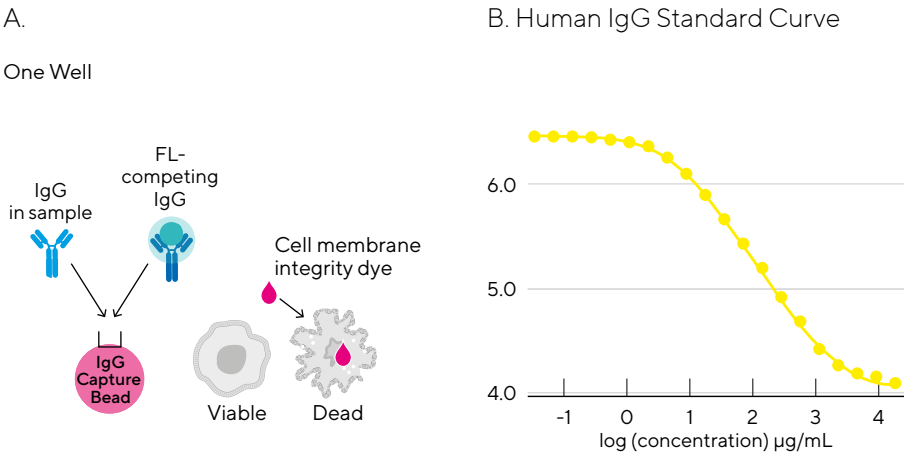


Figure 13: (A) Principle of the iQue® Human Titer and Viability. Fluorescently labeled IgG (FITC-IgG) is added to samples containing secreted IgG and CHO production cells. The FITC-IgG and non-labeled sample IgG compete for binding to IgG capture beads. Cell viability is simultaneously measured in each well using a membrane impermeable integrity dye. (B) IgG concentration is inversely proportional to intensity of fluorescence signal.

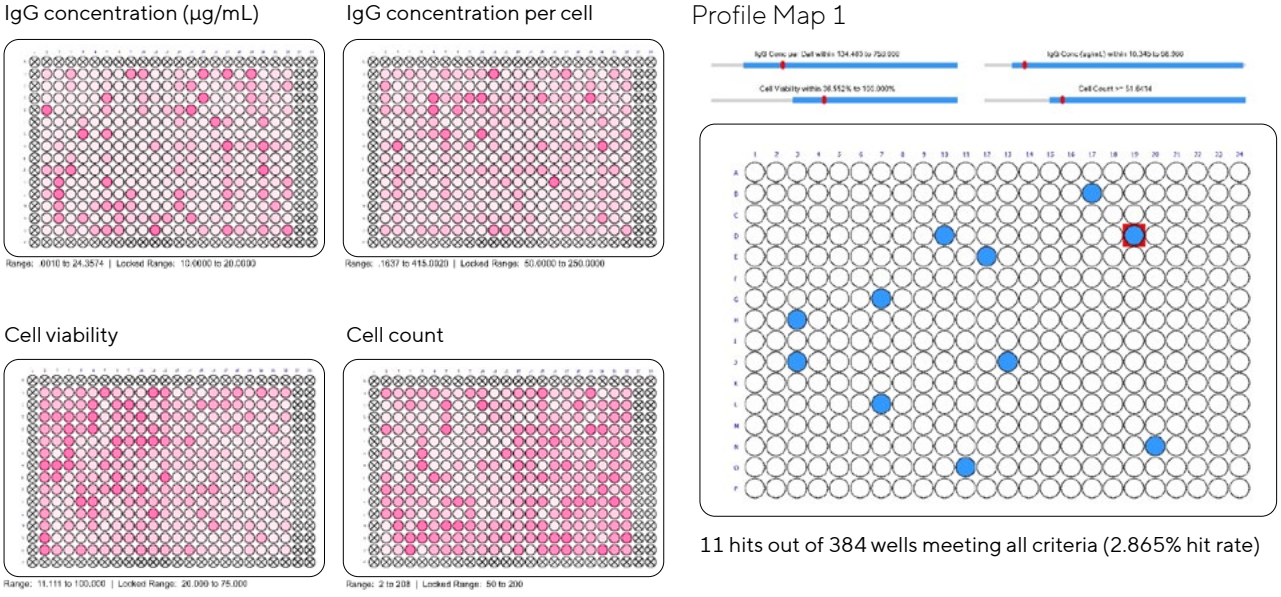


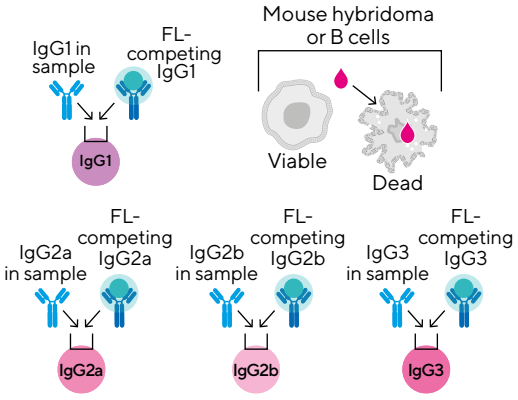
Figure 14: Correlate IgG concentration, cell number, and cell viability in a single well. The customizable profile map feature in iQue Forecyt® allows the user to easily identify hits that meet all the desired selection criteria.

Find out more

iQue® Mouse IgG Type and Titer Assay

Expedite your antibody discovery with this simple, no-wash assay that enables the simultaneous quantitative measurement of each mouse IgG isotype, cell number, and viability from each well of the screening plate in under 2 hours.

A.



B. Representative Standard Curves (Mouse IgG1, 2a, 2b, and

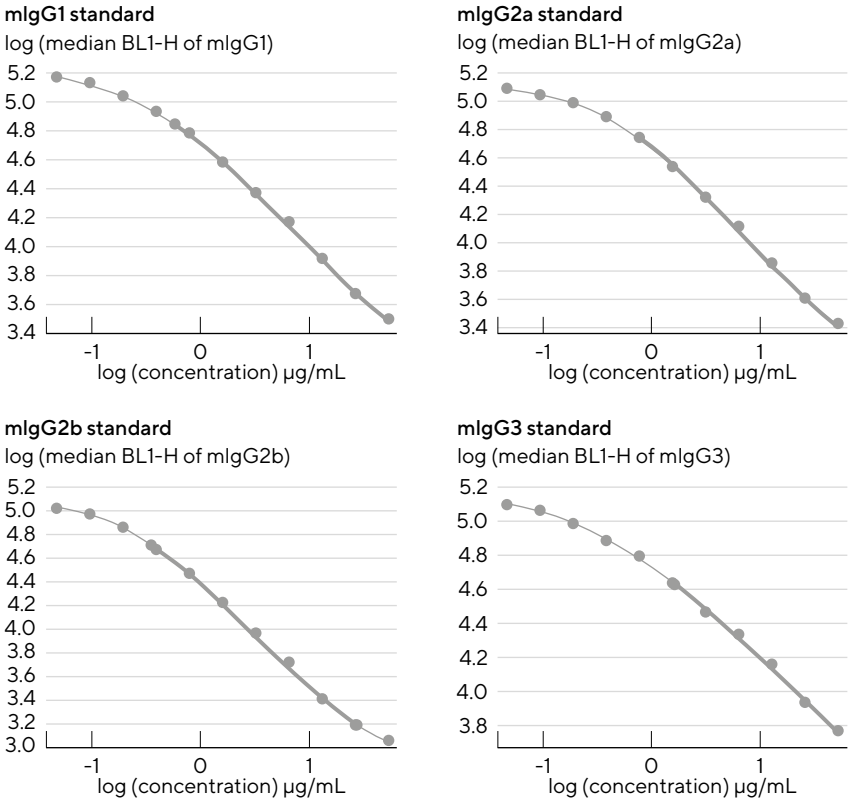
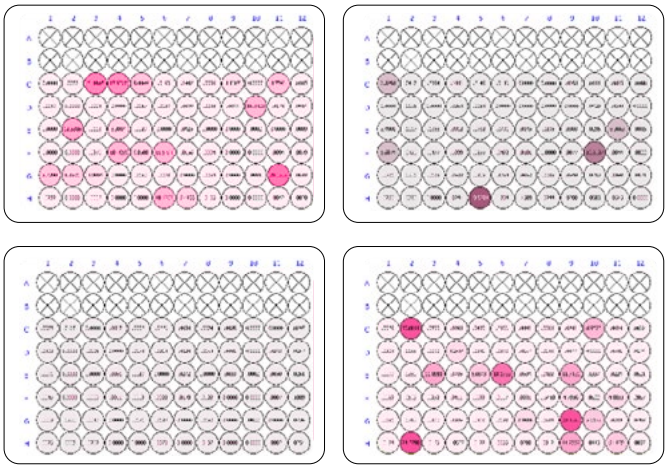


Figure 15: (A) iQue® Mouse IgG Type and Titer Kit assay principle. The no-wash competition assay functions on the differential binding of cell-secreted IgG vs. mouse FITC-IgG to four isotype specific IgG Capture Beads. IgG concentration is inversely proportional to intensity of fluorescence signal. Cell viability is measured simultaneously in each well using a cell membrane impermeable integrity dye. (B) IgG concentration across four isotype-specific beads, in each well are automatically calculated from standard curves for each isotype using iQue Forecyt®.

IgG Quantity



Profile Map — Mouse IgG1

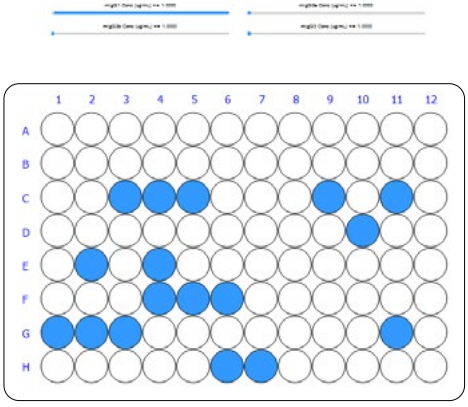


Figure 16: iQue Forecyt® allows simple creation of heat maps to visualize individual isotype secretion trends or customizable profile maps to easily identify wells with desired secretion profiles.

Find out more

iQue® Human and Mouse Antibody Internalization Reagents

The ability to quickly profile and compare large sets of antibodies and characterize their key attributes, such as antibody internalization, can vastly reduce the time required for candidate generation and expedite the development of potential therapeutic treatments. The iQue® Human and Mouse Antibody Internalization Reagents are no-wash pH sensitive dyes that identify antibody internalization from 20 µL of sample in a simple plate-based format. The mouse and human types are designed for use with antibodies containing either mouse or human Fc regions. The assay features the flexibility to combine other validated reagents for multiplexed, no-wash protocols with high throughput capabilities.

iQue® Human and Mouse Antibody Internalization Kits

The iQue® Human and Mouse Antibody Internalization Kits are high throughput, multiplex, no-wash assays that measure antiL assay volume. Antibodies are easily labeled with the pH-sensitive iQue® Antibody Internalization Reagent (R/Red). Cell viability is also measured within the same assay using iQue® Cell Membrane Integrity Dye (B/Green) to assess general cell health and antibody function. Using the same sample, cell specificity can be characterized using the included iQue® Cell Proliferation and Encoding Dye V/Blue (Tag-it Violet™).

Antibody Internalization Reagent Principles

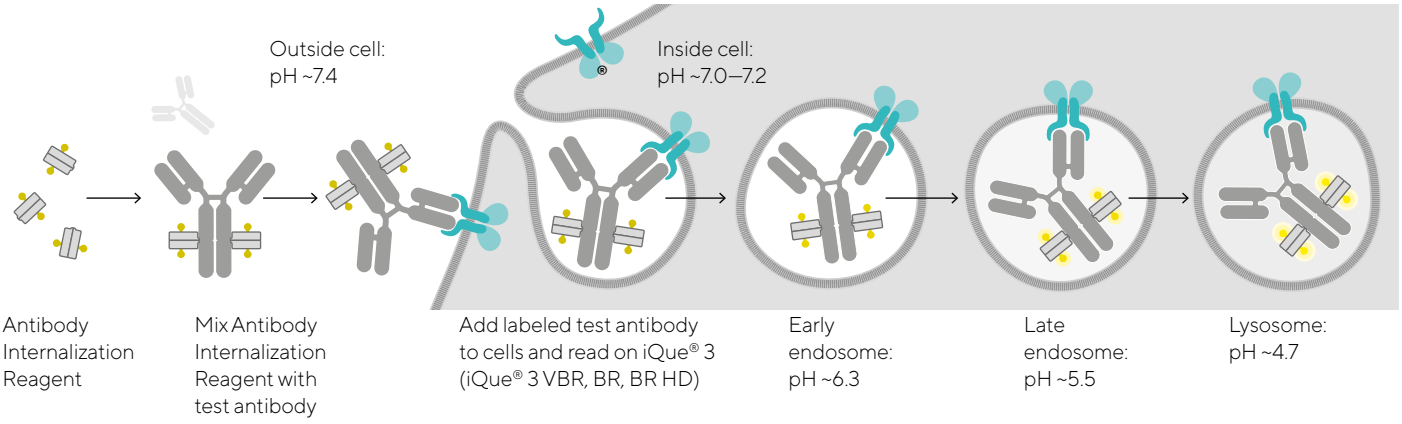
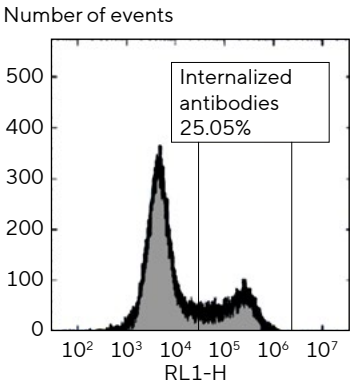


Figure 17: Assay principle of the iQue® Human and Mouse Antibody Internalization Reagents and Kits. Antibodies labeled with the Antibody Internalization Reagent have little fluorescence at neutral pH but become highly fluorescent at a lower pH when they are internalized and processed through the acidic lysosome | endosome pathway.

A. Single Cells



B. Percent Internalized

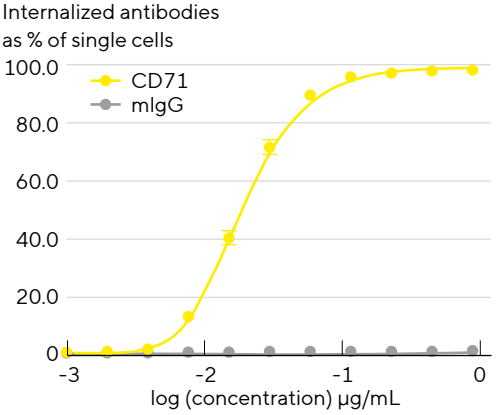


Figure 18: (A) Pre-set template gates are provided for different populations (cells, single cells, and internalized antibodies). The template also provides a dose response curve plotted for MFI and percent internalized for a control sample. Additional curves for further samples can be generated, including (B), the number of cells with internalized antibodies as a percentage of single cells.

Find out more

General Cell Health and Analysis

iQue® Cell-Based Kits

iQue® Cell-Based Kits enable the analysis of multiple cell health and cell function endpoints, such as viability, proliferation, apoptosis, and more. Most of these kits are optimized with no-wash, mix, and read protocols, and can be multiplexed with other iQue® cell or bead-based kits.

Apoptosis

iQue® Human 4-Plex Apoptosis Kit

The no-wash iQue® Human 4-Plex Apoptosis Kit allows the simultaneous detection of Caspase 3/7 activation, Annexin V binding, cell viability, and mitochondrial depolarization from a single sample (Figure 19), in addition to total cell count to identify overly toxic treatments. All four reagents can be run simultaneously (Figures 20 (A) and 20 (B)),or individual reagents are available separately to mix and match according to experimental objectives.

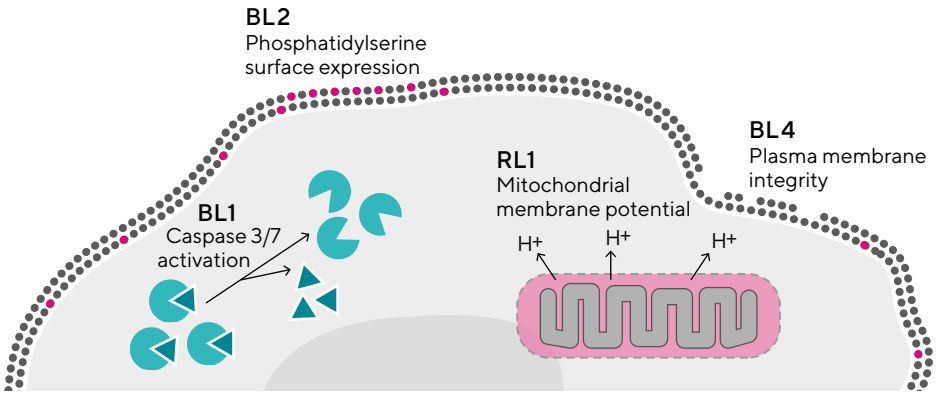


Figure 19: Principle of the iQue® Human 4-Plex Apoptosis Kit. (BL1): Activation of Caspase 3/7 is detected following cleavage by an activated enzyme. (BL2): Surface expression of phosphatidylserine is detected by the binding of Annexin V. (BL4): Cell viability is determined by the uptake of membrane impermeable dye through compromised (porous) membranes. (RL1): Mitochondrial membrane potential is determined by a dye that localizes in the mitochondrial lumen when mitochondria are healthy and able to maintain a membrane potential. Upon mitochondrial depolarization, the dye leaks into the cytoplasm and loses its ability to fluoresce.

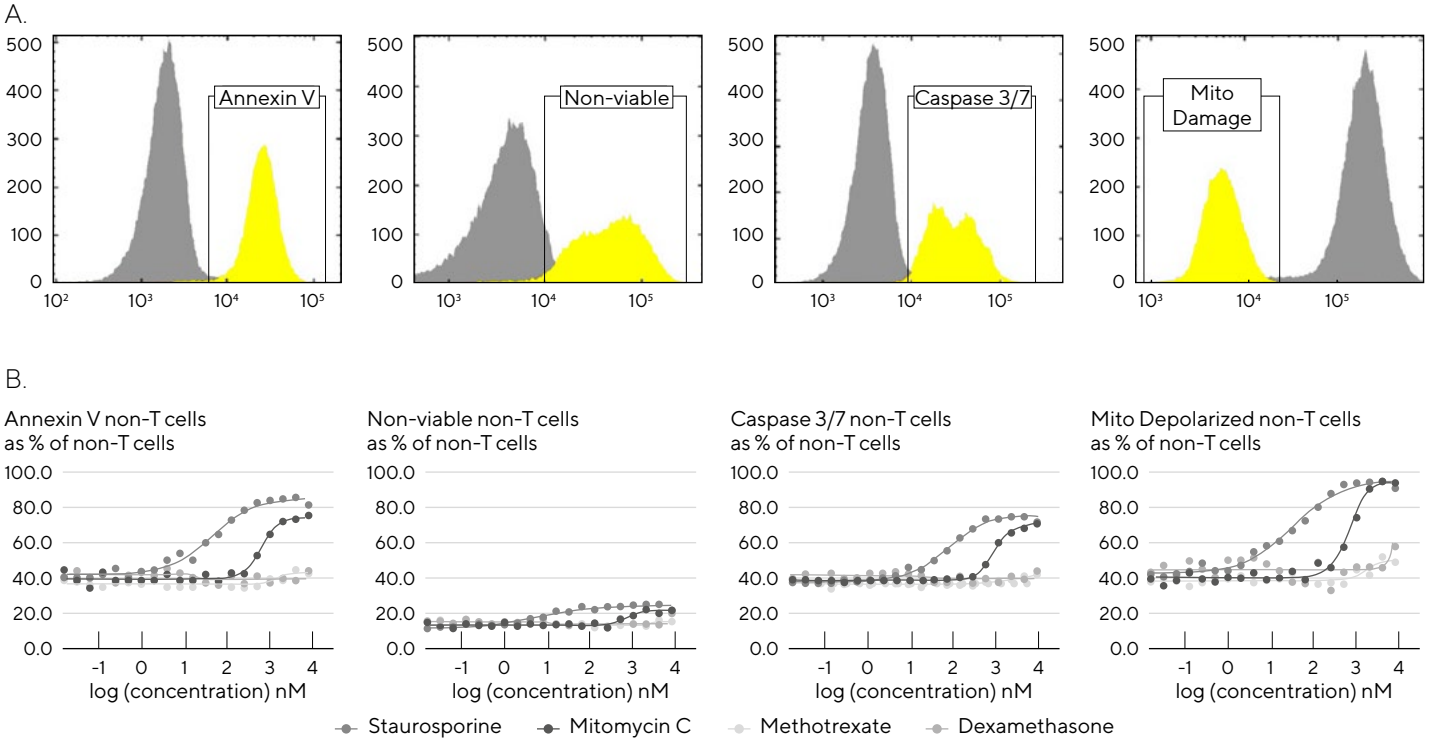


Figure 20: (A) Four distinct hallmarks of cell death enable identification of apoptosis pathways. (B) Compound dose response curves to the addition of Stauropsprorine, Mitomycin C, Methotrexate and Dexamethasone. Histograms and dose response curves generated in iQue Forecyt®.

iQue® Cell Cycle Kit

The iQue® Cell Cycle Kit uses a fluorescent dye that intercalates into DNA, reporting content with enough sensitivity to distinguish between the G0 | G1, G2 | M and S phases (Figure 21).

Unlike traditional methods, the kit requires no wash steps, and the live cell stain can be added without the need to permeabilize, fix or perform an RNase treatment, requiring only a single, 1 hour incubation.

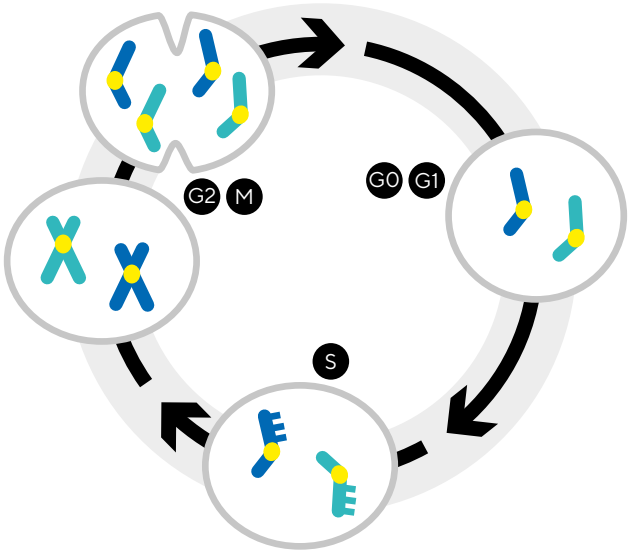


Figure 21: Determining cell cycle stage by DNA content

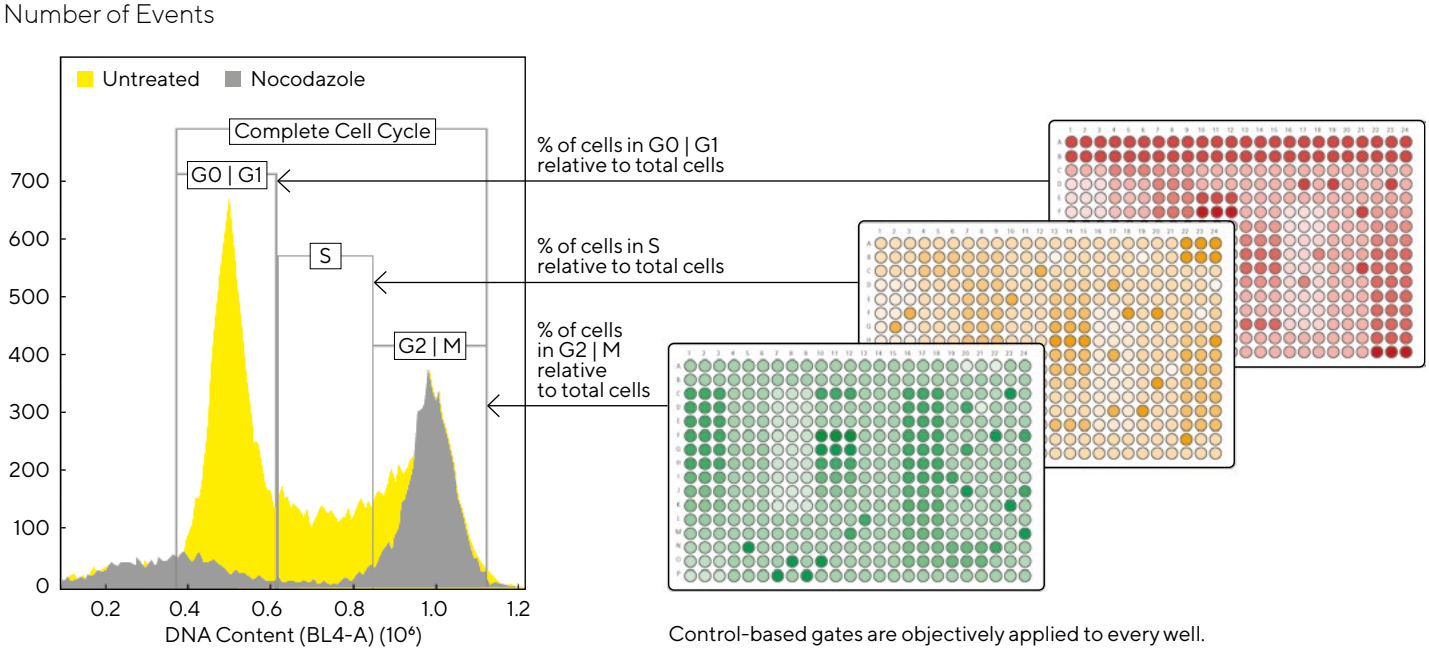


Figure 22: The effects of the cell cycle inhibitor, Nocodazole (gray histogram), can be compared to untreated cells (yellow histogram) using the iQue® Cell Cycle Kit. The percentage of cells in each cycle (G0 | G1, G2 | M, and S) can be quickly compared across multiple plates using heat maps feature in the iQue Forecyt® software.

iQue® Cell Membrane Integrity Dyes

iQue® Cell Membrane Integrity Dyes are comprised of membrane-impermeable, proprietary reagents able to determine cell viability using reagent exclusion and cell membrane integrity as a measurement of cell health (Figure 23). Available in four distinct excitation and emission ranges that enable flexible multiplexing with additional iQue® reagents, the iQue® Cell Membrane Integrity Dyes also offer users a no-wash assay workflow, minimal cytotoxicity up to 48 hours after reagent addition, and robust signal stability with optimized titrations. The B | Red reagent dye will also remain fluorescent for up to 18 hours after fixation in 4% paraformaldehyde (PFA) and is compatible with adherent cells, such as HeLa and A459.

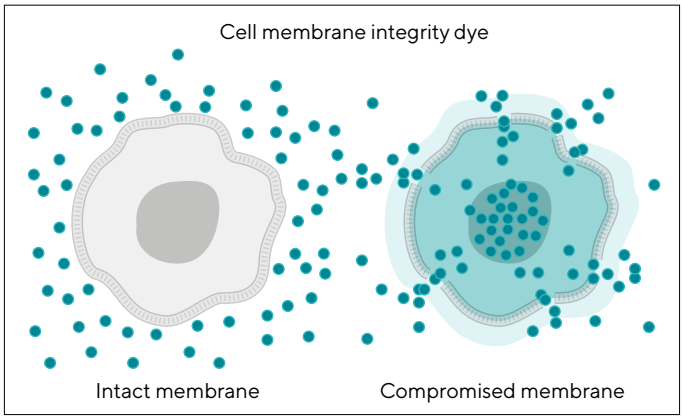
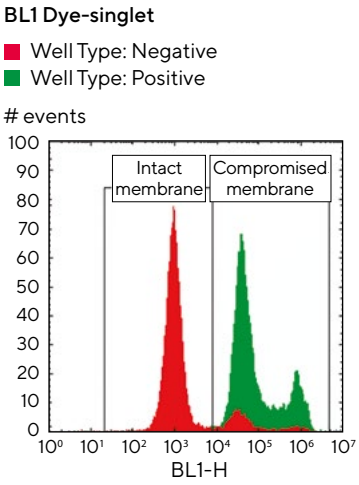


Figure 23: iQue® Cell Membrane Integrity Dye Assay Principles: Cells with intact membranes are able to exclude the cell impermeable reagents and remain non-fluorescent. Once the membranes become compromised, the reagent enters the cell and binds to DNA by intercalation, creating a detectable fluorescent signal.

Representative Data for B | Green Cell Membrane Integrity Reagent



Representative Data for B | Red Cell Membrane Integrity Reagent

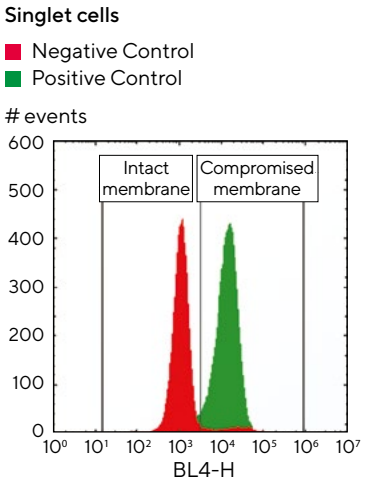


Figure 24: Example readouts to identify cell populations with intact membranes and compromised membranes

Find out more

iQue® Cell Proliferation and Encoding Dyes

The iQue® Cell Proliferation and Encoding Dyes are comprised of proprietary, spectrally distinct, cell permeable dyes that fluoresce after binding to either primary amine groups or glutathione, respectively. With minimal cytotoxicity and increased stability for long term studies up to six generations of proliferated cells can be observed, with no fluorescence intensity gaps between the first and second generation of cells. Alternatively, when used for encoding applications, the iQue® Cell Proliferation and Encoding Dyes offer a robust and flexible solution for the labeling (encoding) of 2 to 4 different cell populations at different intensities in a single fluorescent channel. Each dye is sold individually in several standard sizes and has both wash | no-wash and with | without standard protocols. Available in three distinct excitation | emission ranges, both dyes can be used for either cell proliferation or cell encoding, and can be multiplexed with other iQue® reagents.

	Dead Cells	Latent Cells	Proliferating Cells
Generation 0			
Generation 1			
Generation 2			

Figure 25: Assay Principles for the iQue® Cell Proliferation Dye. Proliferating cells will have decreasing amounts of dye, corresponding to lower fluorescence intensities. Dead or latent cells will maintain the initial dye intensity, which enables easy discrimination between proliferated and non-proliferated cells.

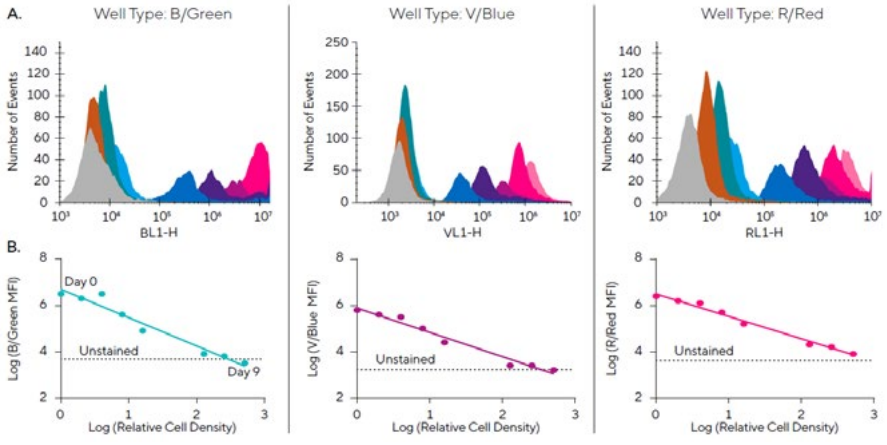


Figure 26: B/Green, V/Blue and R/Red iQue® Cell Proliferation and Encoding Dye stained Jurkats monitored over 10 days, passaging every 48 hours. A) Histograms produce distinct daily peaks until the dye is completely lost. B) Plotting the median fluorescence intensity (MFI) against the relative cell density in a log-log graph shows a linear correlation between the two parameters between day 1 and 5, but the MFI almost matches that of the unstained population by day 9.

Application Spotlight: Immune Cell Killing

Immune cell recognition and killing of unwanted target cells, such as emergent tumor cells, is a critical component of the human host defense mechanism. iQue® kits include optimized reagents that are validated on the iQue® platforms for immune cell killing application areas:

- Adoptive T Cell Therapy
- Chimeric Antigen Receptors
- Tumor Infiltrating Lymphocytes
- NK Cells
- Soluble T Cell Engager

The flexibility of multiplexing with other iQue® kits for further analysis and richer content offers the potential to gain additional insights into the mechanisms of immune cell killing. Monitor viability in both target and effector cells using iQue® Cell Membrane Integrity Dyes, differentiate cells using the iQue® Encoding Dyes, and detect apoptosis in response to immune cell killing (Figures 27 and 28).

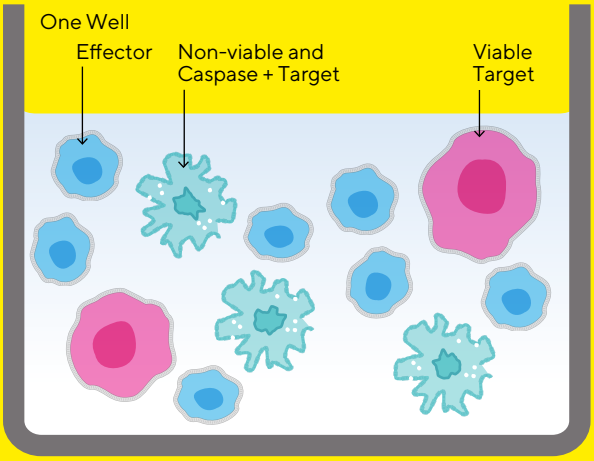


Figure 27: Distinguish target cells from effector cells using the iQue® Encoding Dye. Label dead cells with the iQue® Cell Membrane Integrity Dye. Add additional reagents to assess apoptosis and proliferation, perform cytokine profiling, and phenotyping.

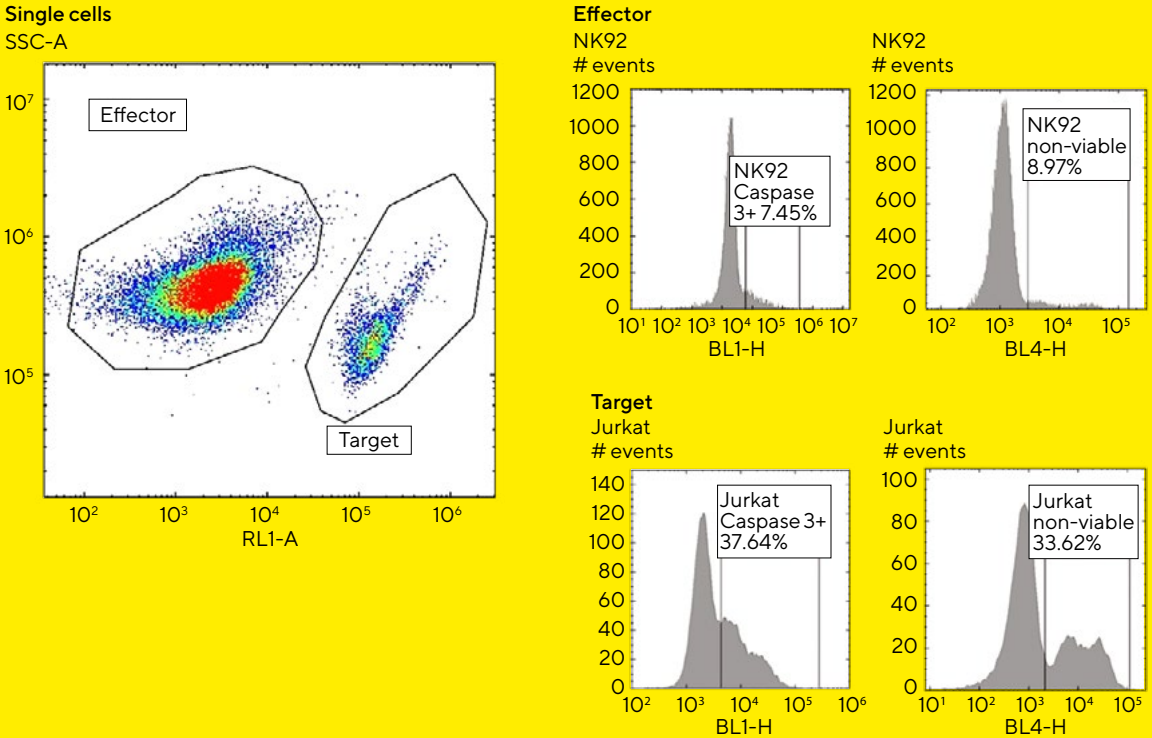


Figure 28: Monitor target cell apoptosis and viability in response to immune cell killing. Independently monitor death in target cells and effector cells using iQue® Encoding Dye, iQue® Caspase 3/7 Kit and iQue® Cell Membrane Integrity Dye, all in a single assay.

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