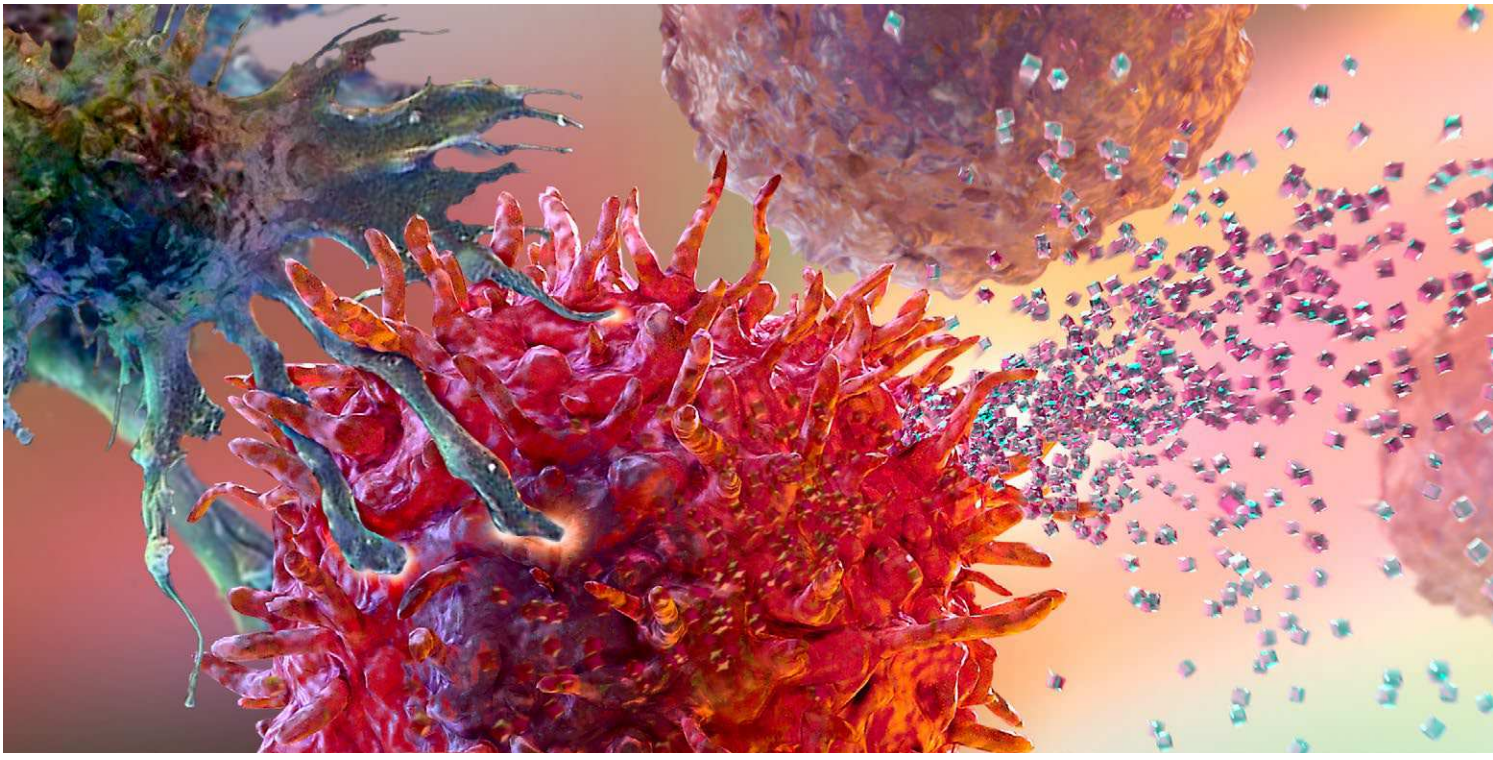
sartorius

High-Throughput, Multiplexed Solutions for Faster Time to Actionable Answers with Intellicyt® Reagent Kits



turning science into solutions

Intellicyt QBeads® and MultiCyt® Reagent Kits

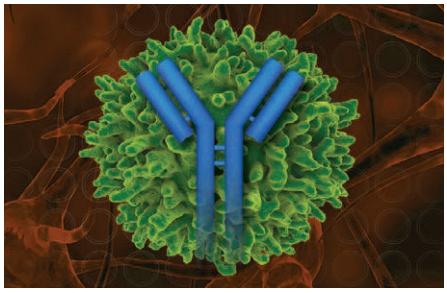
Intellicyt Reagent Kits, available as QBeads, QPanels, and MultiCyt Reagent Kits, provide an integrated solution to enable high content, high throughput flow cytometry based analysis for insight into complex biology. Intellicyt Reagent Kits have been developed to be run on the iQue® platforms and analyzed with ForeCyt® software.

- Biologically relevant results: Multiparametric data gives a fuller understanding of each cell population and more informed actionable results
- Speed: Analyze a 96-well plate in ~5 minutes or a 384-well plate in ~20 minutes for a faster time to answer, with some protocols as little as 100 minutes, including incubation and read time
- Ease of use: A streamlined workflow ensures that more technicians, from novice to expert, can perform assays and maximize the value of the iQue platform in your lab
- Low sample volumes: Use as little as 1 µL, preserving precious sample for additional analysis

QBeads and QPanels allow you to capture and analyze specific proteins on distinct bead types for multiplexed quantitation of cytokines, adhesion molecules, enzymes, growth factor receptors, and more.

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

MultiCyt Reagent Kits



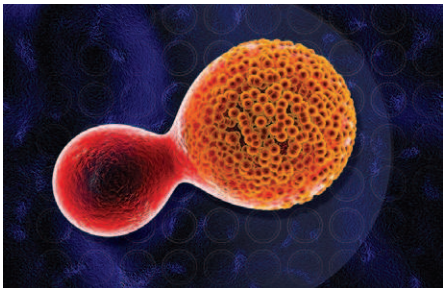
Antibody Discovery and Development

Increase data throughput and quality by multiplexing antibody binding, function, and titer across the process.

Antibody Screening
- Membrane Targets
- Soluble Targets

Functional Profiling
- Proliferation
- Cell Cycle
- Apoptosis
- Antibody Characterization
  - Hybridoma
  - B-Cell
  - Epitope binning
  - Isoforms specificity
  - Species selectivity
  - Internalization
- Immune Function
  - T Cell Activation
  - Cytokine Profiling
- Immune Cell Killing

Cell Line Development
- Clone Selection

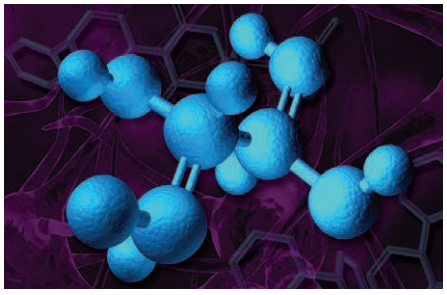


Cell Therapy

Assess multiple cell parameters faster, with fewer cells and less reagents.

Cell Line Engineering/Screening
- Membrane Targets
- Immune Function
  - Immune Cell Killing
  - Phenotyping
  - T Cell Activation
- Cell Health
  - Cytokine Profiling
  - Apoptosis
  - Proliferation

Optimization
- Cytokine Profiling



Small Molecule Screening

Perform high-content phenotypic screening of immune biology across the drug discovery process.

Screening/Optimization
- Cell Health
  - Apoptosis
  - Proliferation
- Cell Cycle
- Immune Function
  - T Cell Activation
  - Phenotyping
  - Cytokine Profiling

Mechanism of Action
- Receptor Activation and Internalization

Kits at a Glance

Kit Type	Key Advantages	Suitable Applications
QBeads PlexScreen[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	Cell characterization, phenotypic screening, and cytokine profiling
QBeads DevScreen[3] Attach your own target proteins or capture antibodies for greatest flexibility	Plex up to 30 of your own analytes with QBeads DevScreen SH, or 5 analytes with QBeads DevScreen SAv	Antibody screening, cell characterization, phenotypic screening, and cytokine profiling
QPanels T Helper Kits[1] Quantify up to 9 analytes in a single sample	Easy to use, with pre-configured, pre-mixed reagents	Cytokine profiling, analysis of T Helper cell secreted cytokines
MultiCyt Immune Cell-Based Kits[1] Assess immune phenotype and function in a single assay	T Cell Activation Kit measures immuno-phenotyping, activation markers, cell health and cytokine profiling markers in a single well Highly reproducible, streamlined workflow with minimal hands-on time	Cytokine profiling, T cell biology, immunophenotyping, and characterization
MultiCyt Cell and Bead-Based Kits[1,2] Screen clones and speed up antibody discovery efforts	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, and cell health
MultiCyt Cell-Based Kits[1,2,3] Understand cell cycle and health, apoptosis, membrane integrity, and proliferation Encode multiple cell lines for multiplexed analysis in a single well	Measure multiple endpoints of cell health and 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

Visit **shop.intellicyt.com** to find and order:

- Reagents and kits based on searches by application, instrument, species reactivity and research areas.
- Detailed information on all of our reagents and kits.

Cytokine Profiling and Bead-based Screening
QBeads and QPanels Kits

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 cytokines, adhesion molecules, enzymes and growth factor receptors using minimal sample volume and a simple, fast workflow. QBeads come in two variations: QBeads PlexScreen Reagents and QBeads DevScreen Kits.

QBeads PlexScreen Reagents

- Custom built, ready-to-run kits used 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.
- Use with other MultiCyt kits, with no reduction in analysis speed.
- Validated no-wash or 1-wash protocols.
- Consist of 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).

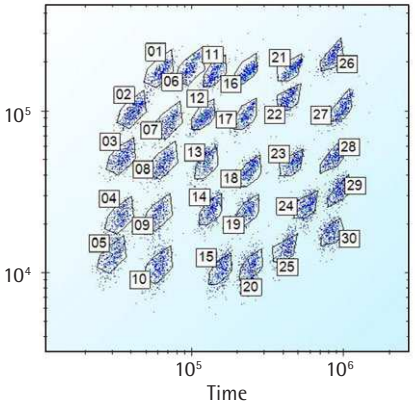


Figure 1. Pre-defined analysis templates are provided with each kit for the included analytes. Bead population gates are empty before acquisition and are populated with the appropriate beads during sample acquisition.

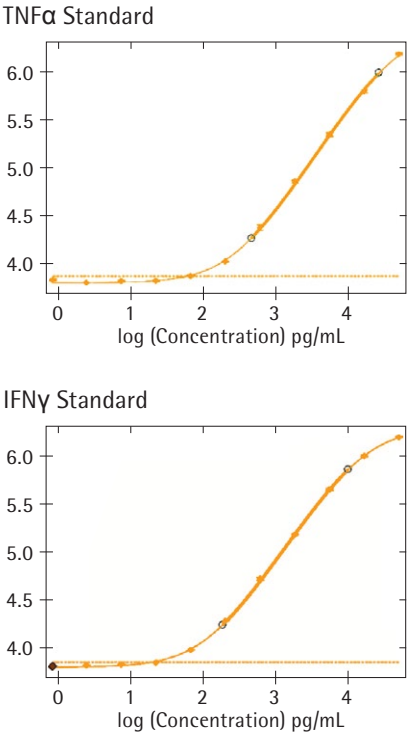


Figure 2. Sample standard curves generated for different cytokines.

intellicyt.com/qbeads-kits/

Please visit **intellicyt.com/qbeads-assay-builder** to build a panel and request a quotation.

QBeads DevScreen Kits

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

- QBeads DevScreen Streptavidin Coated Reagents
  - Streptavidin Coated Kits used to screen biotinylated targets (Figure 3).
  - Available in 5 different bead populations.
  - Multiplex with analytes from the PlexScreen or DevScreen Sulfhydryl (SH) panels.
- QBeads DevScreen Sulfhydryl Derivatized Reagents
  - Sulfhydryl coated beads covalently bind any molecule with a free amine functional group in a simple 2 step process.
  - Available in up to 30 different bead populations.
  - Multiplex with QBeads PlexScreen and DevScreen SAv reagents kits (multiplexing with QBeads PlexScreen is subject to bead compatibility).

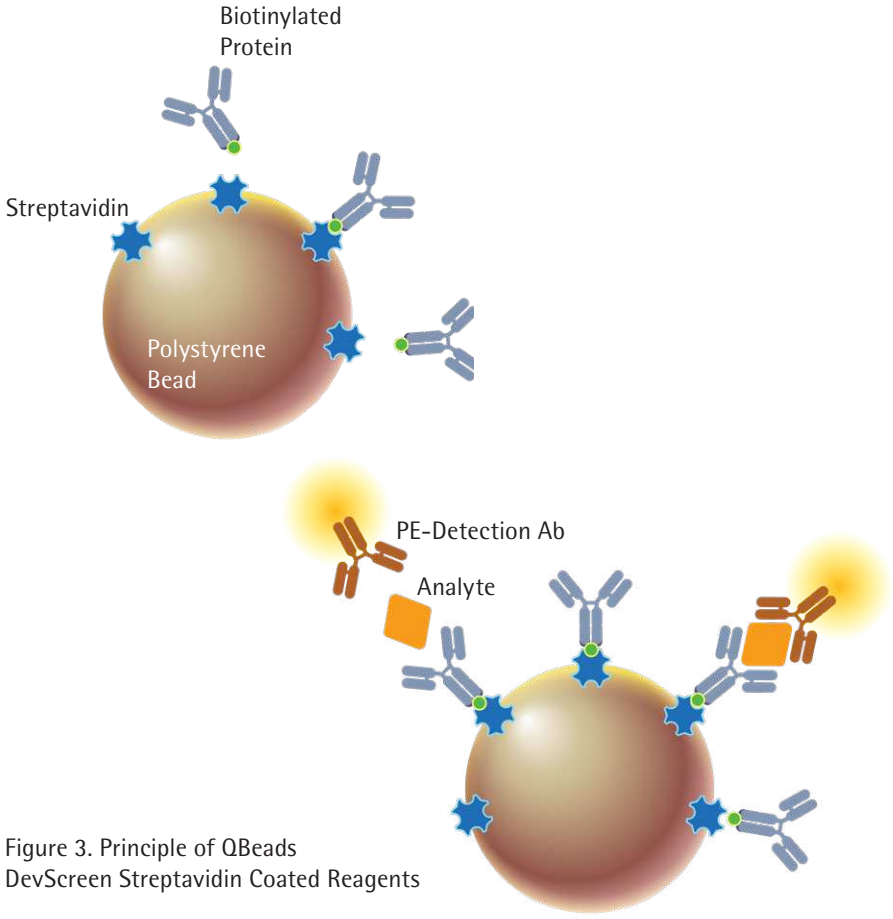


Figure 3. Principle of QBeads DevScreen Streptavidin Coated Reagents

QPanels T Helper Kits

With pre-configured beads for the qualitative and quantitative analysis of up to 9 analytes in a single sample of plasma, serum or cell culture supernatant, QPanels T Helper Kits are designed for multiplexed detection of cytokines secreted by human T Helper cells. Each kit has been optimized for different analyte combinations, specifically designed for ease-of-use with pre-mixed beads and standards, and requires only 10 µL of sample.

QPanels T Helper Kits	Analytes
Th1/2 | 4-plex	IL-4, IL-6, IFNγ, TNFα
Th1/2 | 6-plex	IL-2, IL-4, IL-6, IL-10, IFNγ, TNFα
Th1/2 | 9-plex	IL-2, IL-4, IL-6, IL-10, IL-12 (p70), IL-13, IFNγ, TNFα, GM-CSF
Th1/2/17 | 7-plex	IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ, TNFα

intellicyt.com/qpanels-kits

Immune Cell Function and Characterization
MultiCyt Immune Cell-Based Kits

Human T Cell Activation Cell and Cytokine Profiling Kit

The Human T Cell Activation Cell and Cytokine Profiling Kit greatly streamlines the traditional workflow by measuring immune cell phenotypes, T cell activation markers, cell proliferation, cell viability and quantitates secreted cytokines (IFNγ and TNFα) in a single 5 µl sample using a miniaturized multi-well plate format (Figure 4).

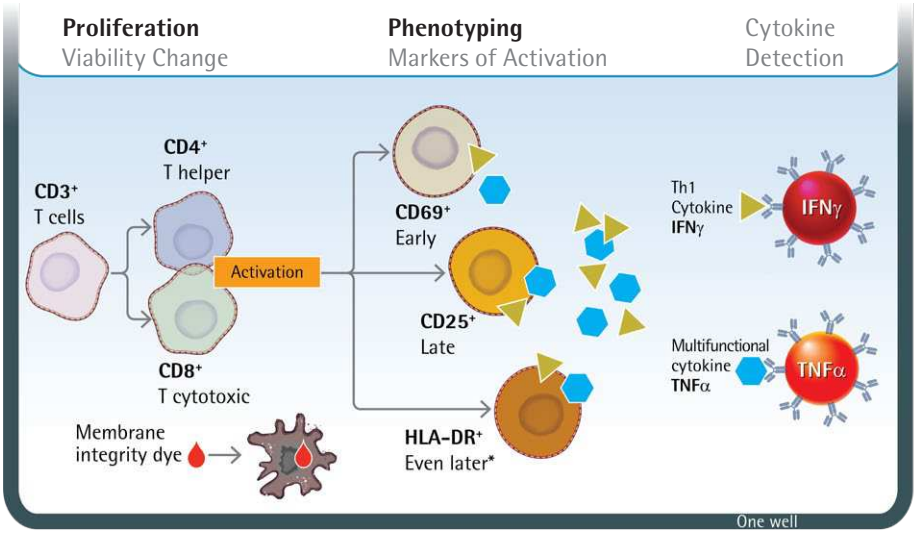


Figure 4. Principle of the Human T Cell Activation Cell and Cytokine Profiling Kit assay. Simultaneous measurement of activation markers, cell proliferation and cytokines in a single well.

Human T Cell Companion Kits

Human T Cell Companion Kits are used in combination with the Human T Cell Activation Cell and Cytokine Profiling Kit and allow the measurement of up to six further human cytokines in addition to those already included in the Human T Cell Activation Cell and Cytokine Profiling Kit. The Human T Cell Companion Kits are supplied with their own pre-formatted analysis template (Figure 5).

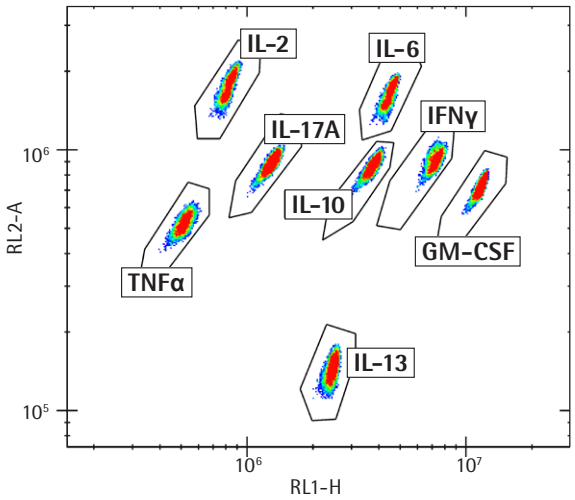


Figure 5. Human T Cell Companion Kit cytokine template.

intellicyt.com/tca

Application Spotlight:
T Cell Activation

The study of immune cell function, such as T cell activation, requires a complete understanding of dynamic changes in immuno-phenotype profiles, cell health and proliferation as well as the relative amounts of various secreted cytokines that are important indicators of activation. Assessment of multiple parameters shown in Figure 6 has traditionally required the use of several platforms followed by time-consuming data merging and interpretation to understand the biology being studied.

The first in class Human T Cell Activation Cell and Cytokine Profiling Kit enables the generation of data for every parameter of activation in a single well (Figure 7). Providing the most biologically relevant information from the smallest amount of sample in the shortest amount of time allows researchers to advance scientific programs faster than ever before.

Cellular Subsets		
CD3	CD4	CD8
Markers of Activation		
CD69	CD25	HLA-DR
Proliferation		
Cytokines		
IFNγ	TNFα	

Figure 6. Markers measured using the Human T Cell Activation Kit.
Note: T Cell Companion Kits can be used in conjunction with the Human T Cell Activation Kit to analyze up to six additional cytokines as shown in Figure 5.

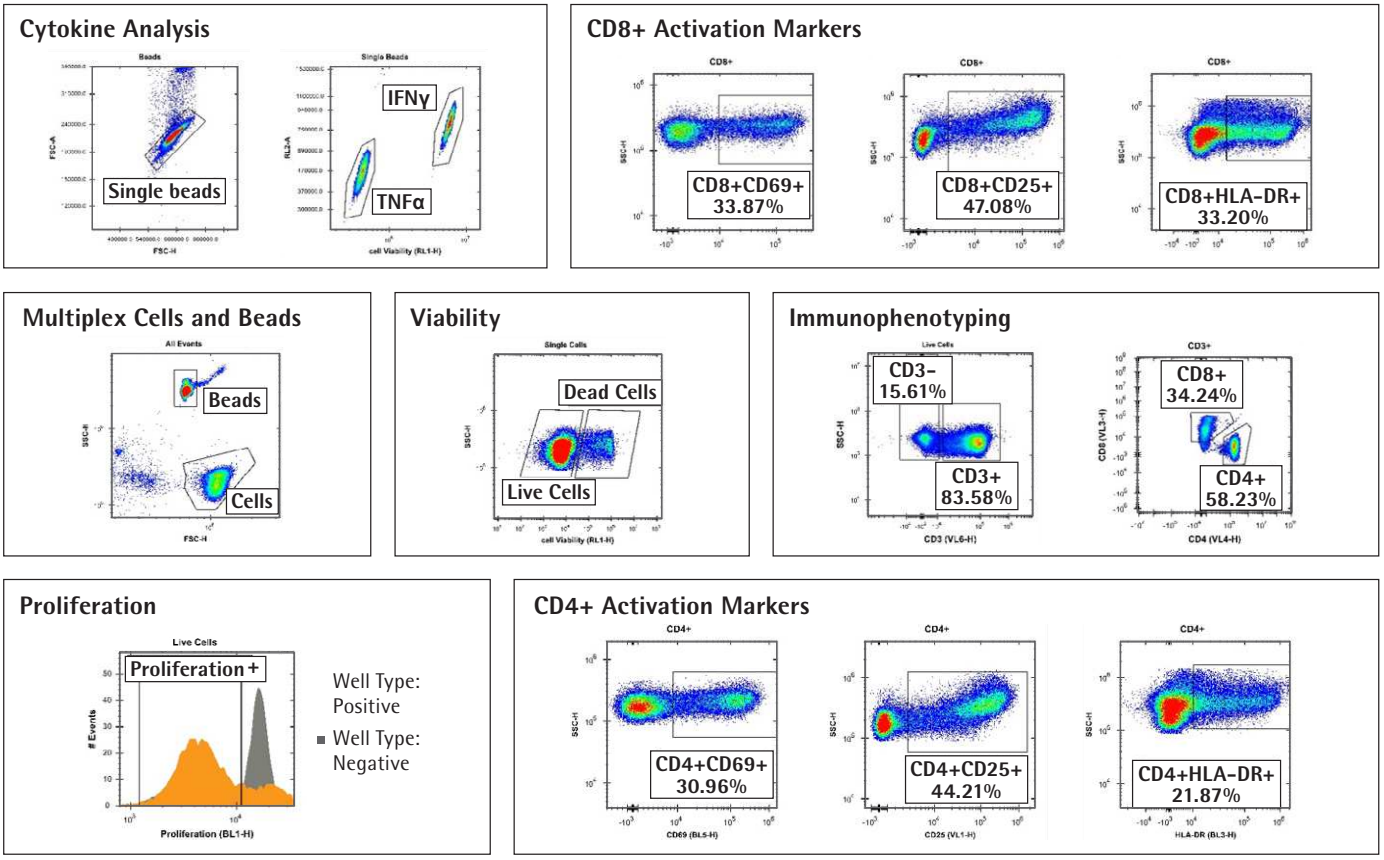


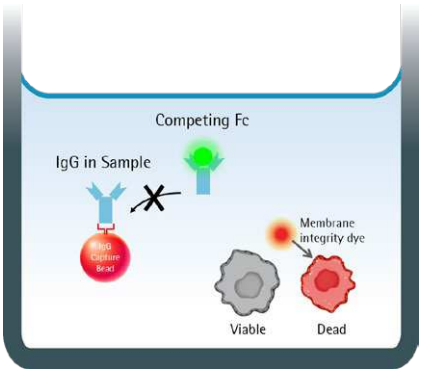
Figure 7. Analysis of the T Cell Activation Cell and Cytokine Profiling Kit. A pre-defined, standardized template makes collection and interpretation of the large quantity of data easy. High content data can be analyzed quickly and efficiently using ForeCyt software. All eleven parameters can be combined to identify hits on a single data graphic, and results from multiple plates can be subsequently analyzed using features included in ForeCyt software.

Cell Line Development and Antibody Characterization
MultiCyt Cell and Bead Based Kits

Cy-Clone™ PLUS Kit

Early identification of the most efficient, high-producing cell lines can significantly increase the probability of success in downstream scale-up activities. 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.

8a.



8b. Human IgG Standard Curve

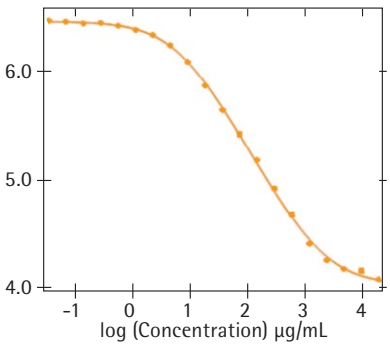


Figure 8a. Principle of the Cy-Clone PLUS Kit. Fluorescently labeled IgG (FITC-IgG) is added to samples containing secreted IgG and CHO production cells. The FITC-IgG and non-labeled sample IgG compete for binding to IgG capture beads. Cell viability is simultaneously measured in each well using a membrane impermeable integrity dye. Figure 8b. IgG concentration is inversely proportional to intensity of fluorescence signal.

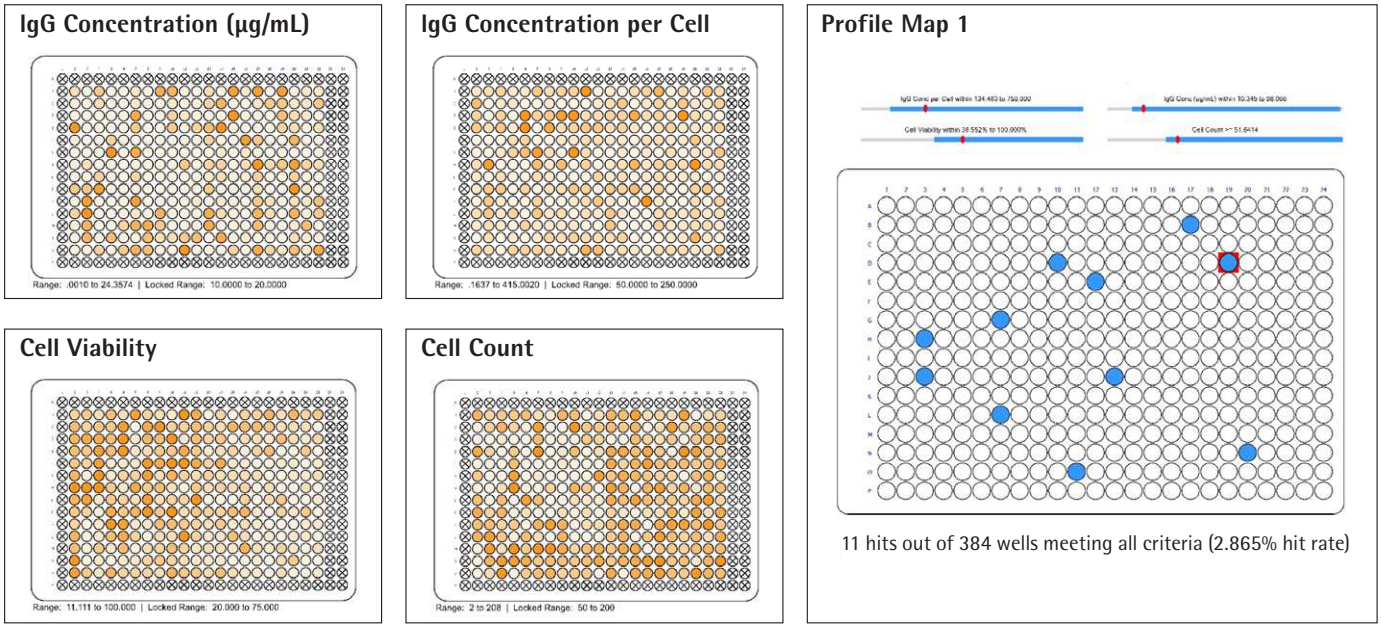


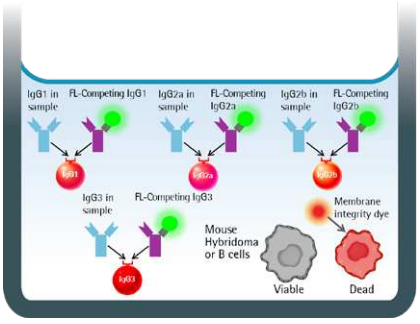
Figure 9. Correlate IgG concentration, cell number and cell viability in a single well. The customizable profile map feature in ForeCyt allows the user to easily identify hits that meet all the desired selection criteria.

intellicyt.com/cy-clone-plus

Mouse IgG Type and Titer Assay

Speed up 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.

10a.



10b. Representative standard curves (mouse IgG1, 2a, 2b, and 3)

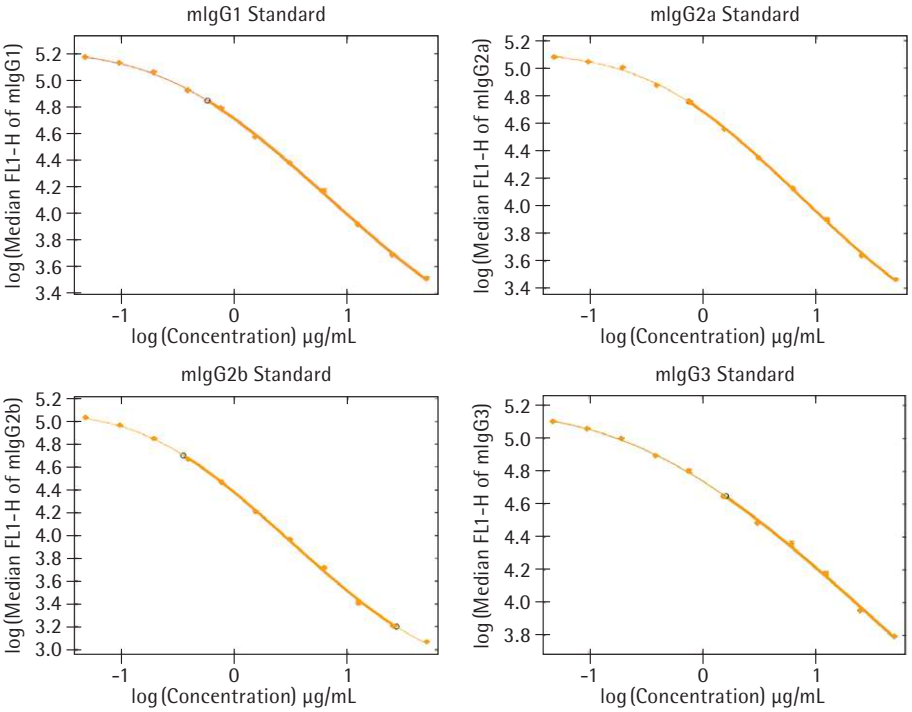


Figure 10a. Mouse IgG Type and Titer Kit assay principle. The no-wash competition assay functions on the differential binding of cell-secreted IgG vs mouse FITC-IgG to four isotype specific IgG Capture Beads. IgG concentration is inversely proportional to intensity of fluorescence signal. Cell viability is measured simultaneously in each well using a cell membrane impermeable integrity dye. Figure 10b. IgG concentration across four isotype-specific beads in each well are automatically calculated from standard curves for each isotype using ForeCyt.

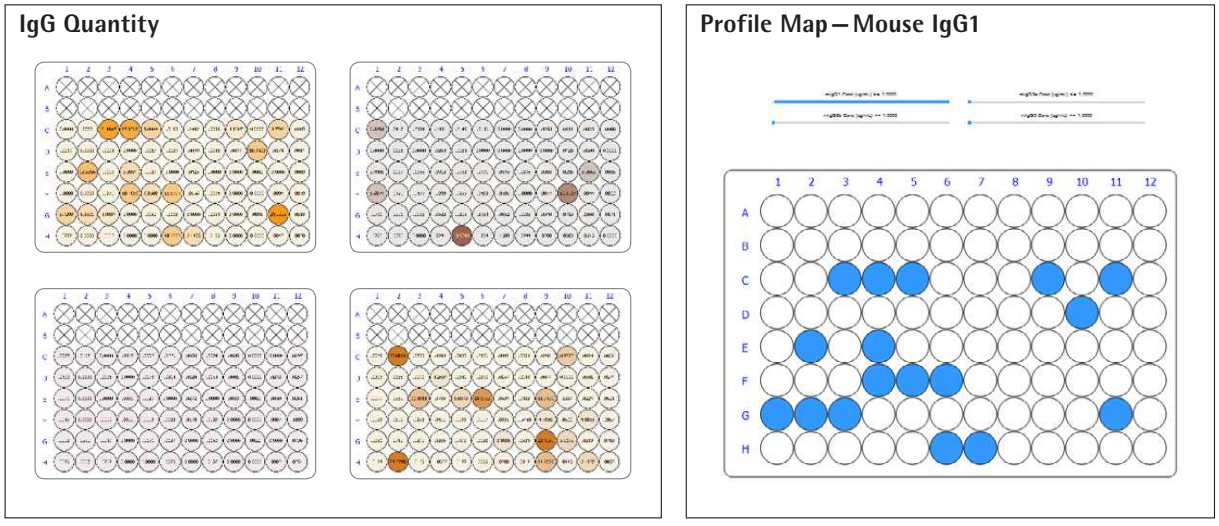


Figure 11. Forecyt allows simple creation of heat maps to visualize individual isotype secretion trends or customizable profile maps to easily identify wells with desired secretion profiles.

intellicyt.com/mouse-igg-kit

Intellicyt Antibody Internalization Reagent

The Antibody Internalization Reagent is a novel, no-wash pH sensitive dye that identifies antibody internalization from 20 µL of sample in a simple plate-based format. 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.

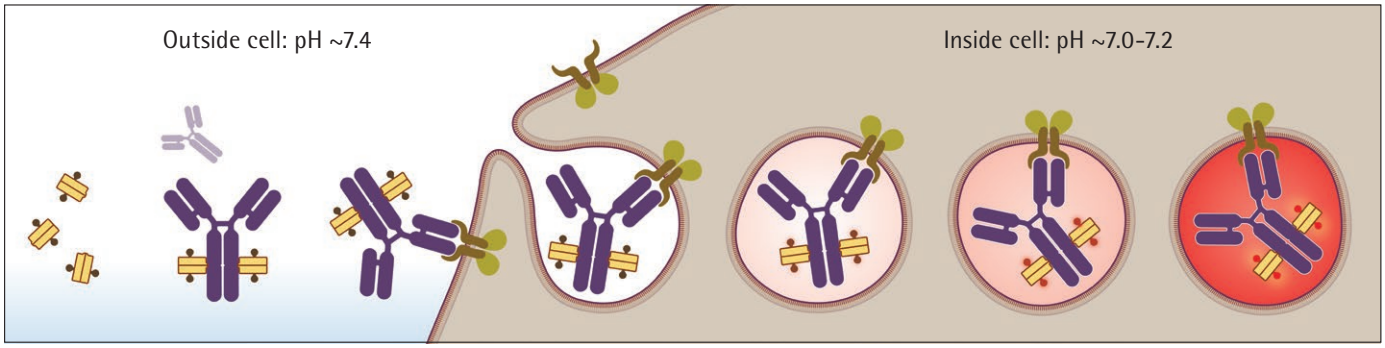
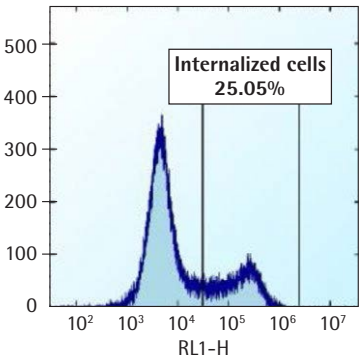


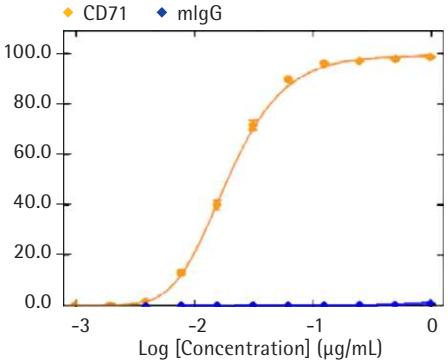
Figure 12. Assay principle of the Antibody Internalization Reagent. Antibodies labeled with the Antibody Internalization Reagent have little fluorescence at neutral pH but become highly fluorescent at a lower pH when they are internalized and processed through the acidic lysosome/endosome pathway.

Figure 13a. Pre-set template gates are provided for different populations (cells, single cells and internalized cells). 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 Figure 13b, the number of internalized cells as a percentage of single cells.

13a. Single cells
Number of events



13b. Percent internalized
Internalized cells as % of single cells



intellicyt.com/multicyt-kits

General Cell Health and Analysis
MultiCyt Cell Based Kits

MultiCyt 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 MultiCyt cell or bead-based kits.

APOPTOSIS

MultiCyt 4-Plex Apoptosis Kit

The no wash 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 14), in addition to total cell count to identify overly toxic treatments. All four reagents can be run simultaneously (Figures 15a and 15b), or individual reagents can be "mixed and matched" according to experimental objectives.

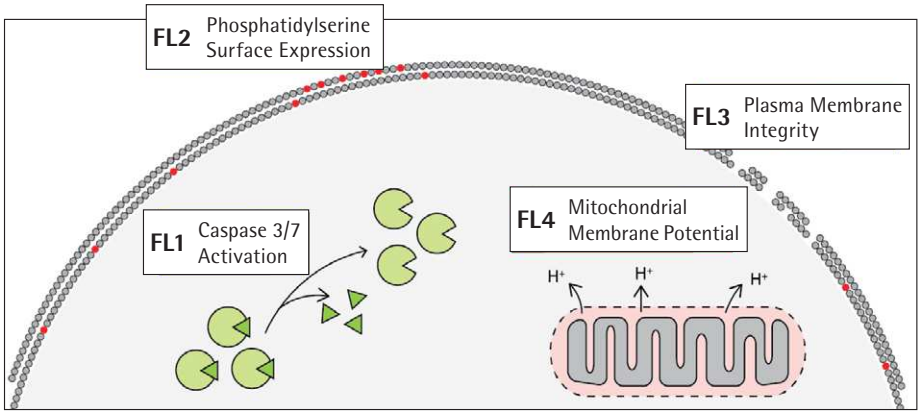
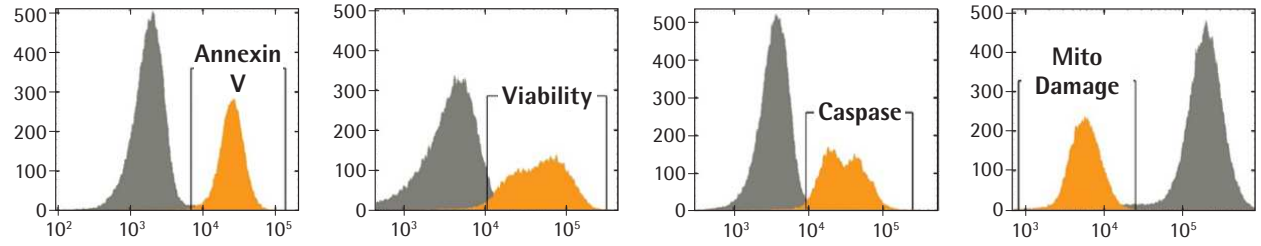


Figure 14. Principle of the MultiCyt 4-Plex Apoptosis Kit. (FL1): Activation of Caspase 3/7 is detected following cleavage by an activated enzyme. (FL2): Surface expression of phosphatidylserine is detected by the binding of Annexin V. (FL3): Cell viability is determined by the uptake of membrane impermeable dye through compromised (porous) membranes. (FL4): 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.

15a.



15b. Caspase 3/7+ non-T cells as % of non-T cells

Annexin V+ non-T cells as % of non-T cells

Viability non-T cells as % of non-T cells

Mito Depolarized non-T cells as % of non-T cells

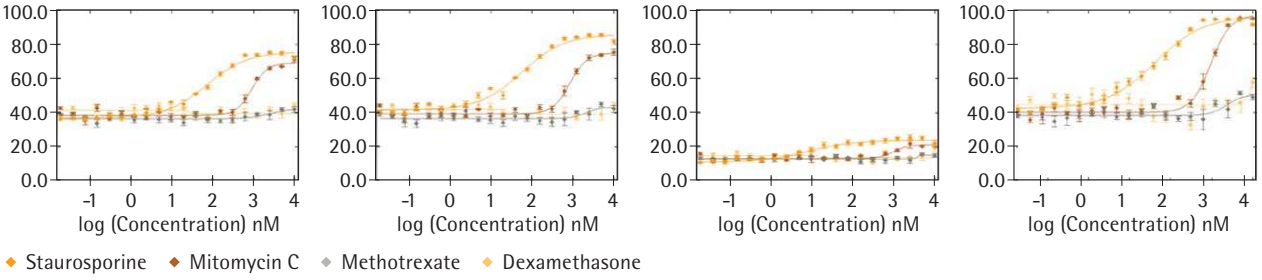


Figure 15a. Four distinct hallmarks of cell death enable identification of apoptosis pathways. Figure 15b. Compound dose response curves to the addition of Stauropsprorine (orange), Mitomycin C (brown), Methotrexate (grey) and Dexamethasone (yellow). Histograms and Dose Response Curves generated in ForeCyt.

MultiCyt Cell Cycle Kit

The Cell Cycle Staining 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 16).

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.

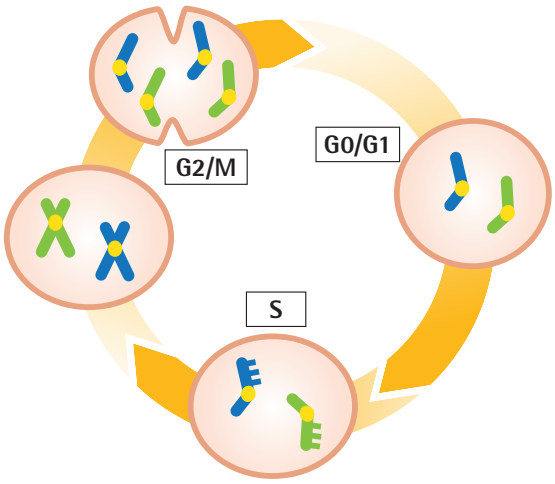


Figure 16. Determining cell cycle stage by DNA content.

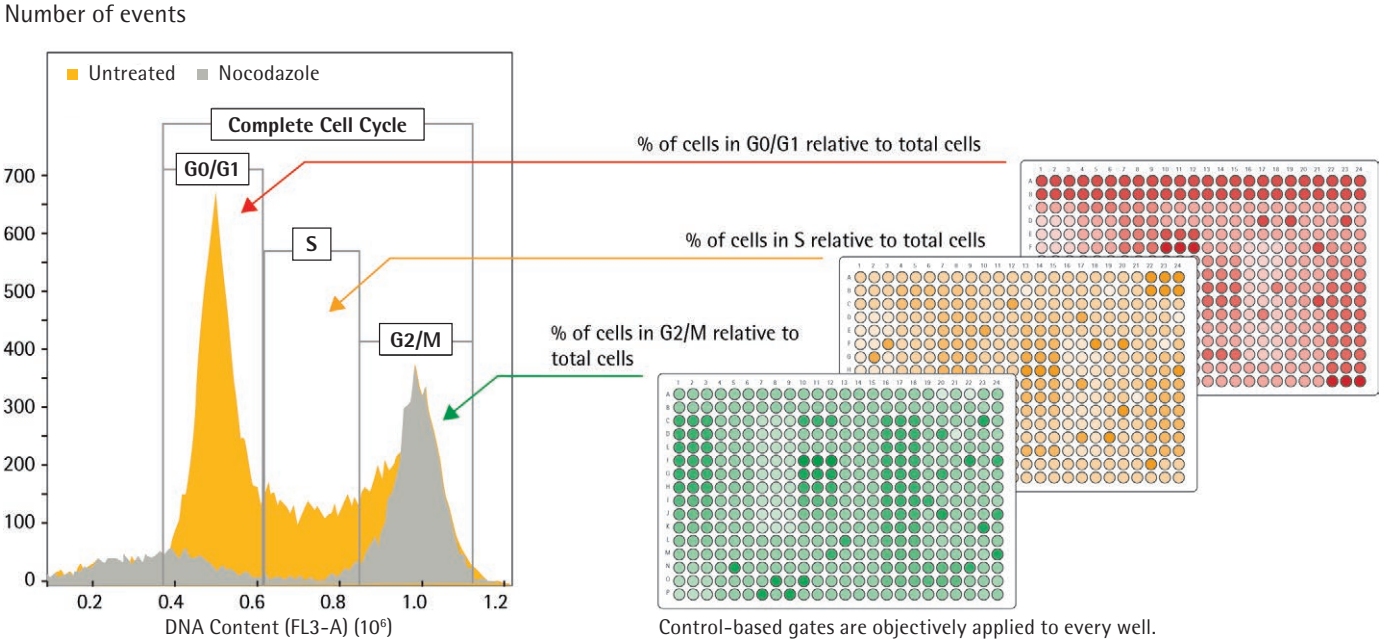


Figure 17. The effects of the cell cycle inhibitor, Nocodazole (grey histogram), can be compared to untreated cells (yellow histogram) using the MultiCyt Cell Cycle Kit. The percentage of cells in each cycle (G0/G1, G2/M and S) can be quickly compared across multiple plates using heat maps feature in the ForeCyt software.

MultiCyt Cell Membrane Integrity Kits

MultiCyt Cell Membrane Integrity Kits 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 18). Available in three distinct excitation and emission ranges that enable flexible multiplexing with additional MultiCyt reagents, the MultiCyt Cell Membrane Integrity Panel also offers 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 up to 18 hours after fixation in 4% paraformaldehyde (PFA) and is compatible with adherent cells, such as HeLa and A459.

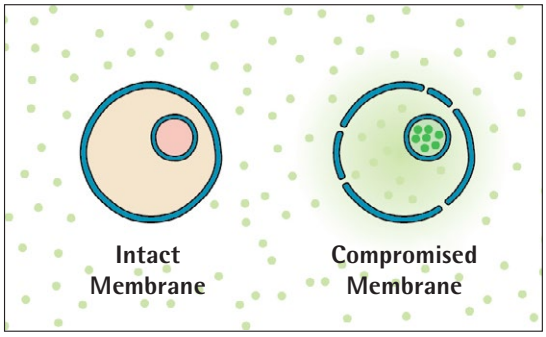
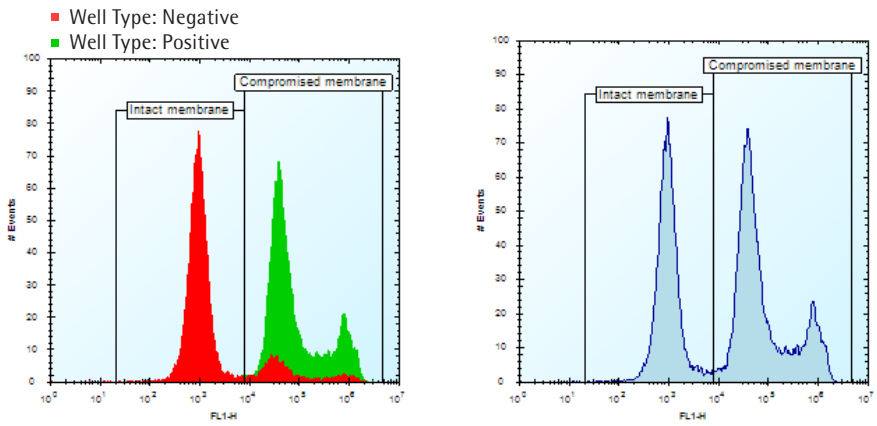


Figure 18. MultiCyt Cell Membrane Integrity Dye Assay Principles: Cells with intact membranes are able to exclude the cell impermeable reagents and remain non-fluorescent. Once the membranes become compromised, the reagent enters the cell and binds to DNA by intercalation, creating a detectable fluorescent signal.

Representative data for B/Green cell membrane integrity reagent



Representative data for B/Red cell membrane integrity reagent

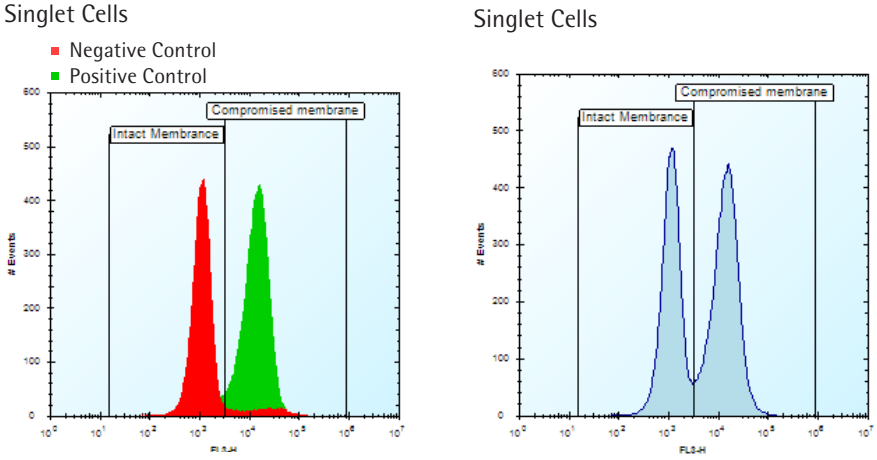


Figure 19. Example readouts to identify cell populations with intact membranes and compromised membranes.

intellicyt.com/multicyt-kits

MultiCyt Cell Proliferation and Encoder Kits

The MultiCyt Cell Proliferation and Encoder Kits 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 MultiCyt Cell Proliferation and Encoder Kits 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 2 distinct excitation/emission ranges, both dyes can be used for either cell proliferation or cell encoding and can be multiplexed with other MultiCyt reagents.

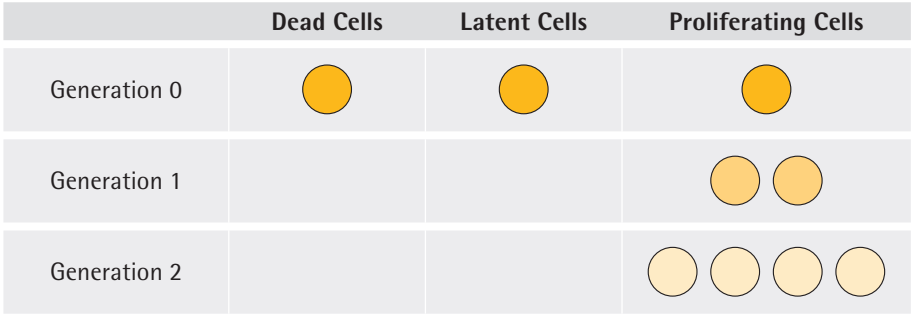
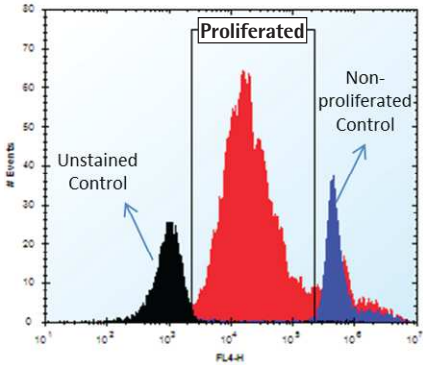
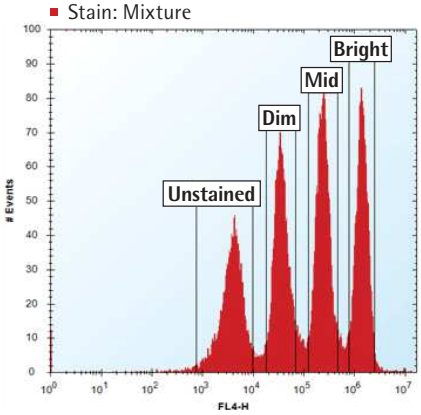


Figure 20. Assay Principles for the MultiCyt Cell Proliferation Kit. Proliferating cells will have decreasing amounts of dye, corresponding to lower fluorescence intensities. Dead or latent cells will maintain the initial dye intensity, which enables easy discrimination between proliferated and non-proliferated cells.

21a. FL4 Histogram
Singlet cells



21b. FL4-H 1D Histogram
Singlet cell encoder



21c. FL4-H vs FSC-H 2D Plot
1:6 Serially titrated

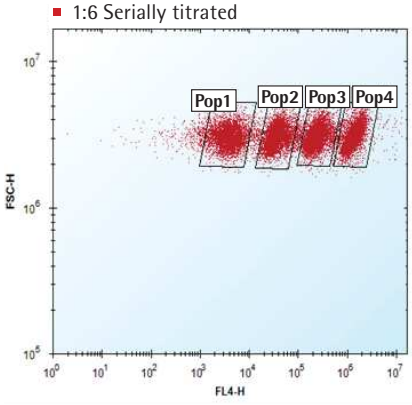


Figure 21a. Identification of the proliferated cell population. Figures 21b and 21c. Identifying the various encoded cell populations. The MultiCyt Cell Proliferation and Encoder reagents have been extensively tested for screening applications using both suspension (PBMC as shown above, Jurkat, Ramos and U937) and adherent (HeLa, A549 and H4) cell lines.

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. MultiCyt 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 MultiCyt 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 Cell Membrane Integrity Kits, differentiate cells using the Encoder Dye Kits and detect Apoptosis in response to immune cell killing (Figures 22 and 23).

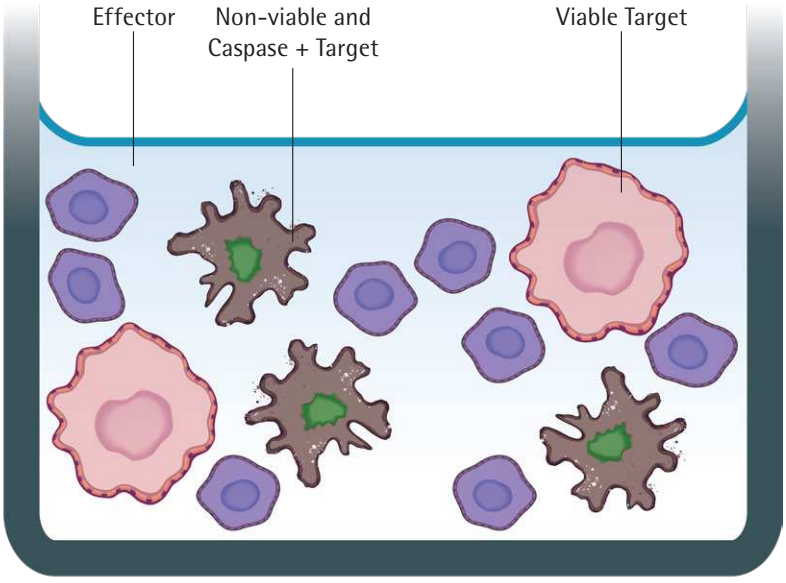


Figure 22. Distinguish target cells from effector cells using the Encoder Dye Kit. Label dead cells with the Membrane Integrity Dye Kit. Add additional reagents to assess apoptosis and proliferation, perform cytokine profiling, and phenotyping.

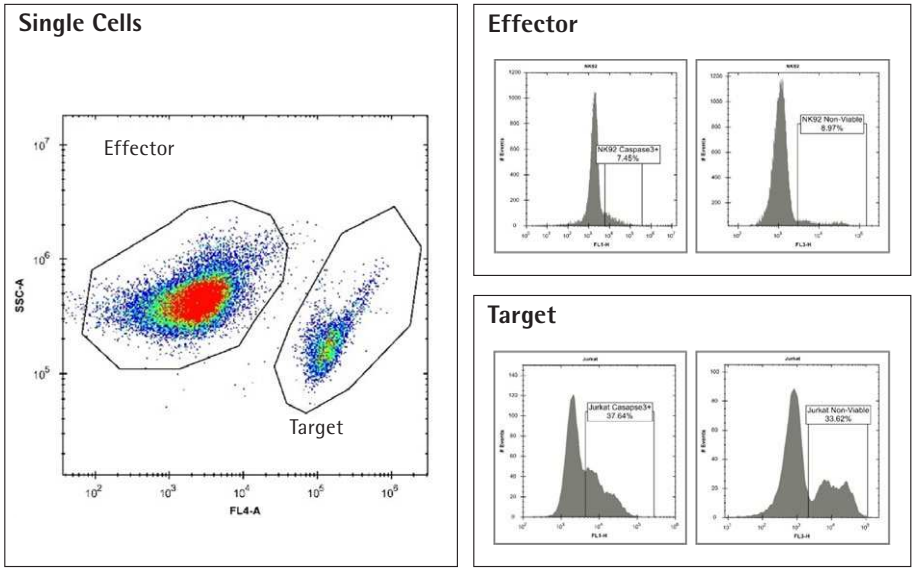


Figure 23. Monitor target cell apoptosis and viability in response to immune cell killing. Independently monitor death in target cells and effector cells using Encoder Dye, Caspase and Membrane Integrity Dye Kits, all in a single assay.

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- Reagents and kits based on searches by application, instrument, species reactivity and research areas.
- Detailed information on all of our reagents and kits.

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