

Automating Flow Cytometry

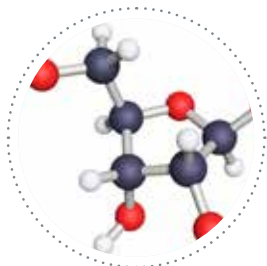


EVERY
event matters.

 **BECKMAN
COULTER**
Life Sciences

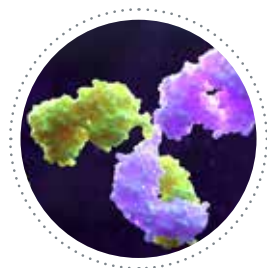
USE OF CELLS IN THERAPEUTIC PRODUCTS DEVELOPMENT

Living cells have become a crucial component in the development of therapeutic products, not only in the biopharmaceutical industry but also in traditional drug discovery programs:



Small Molecule

Cell-based assays are used in Small Molecule drug discovery to gather information on biological activity, toxicology, molecular mechanisms and off-target effects.



Biologic Molecule

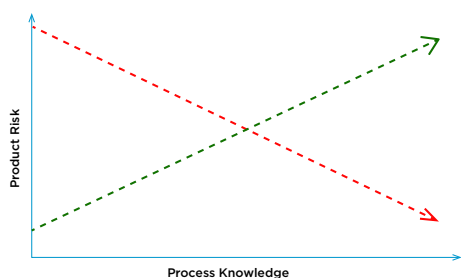
Produced in living cells typically in fed-batch bioreactors. Cell-based assays are critical for immune system monitoring, including effector cell activation, immune checkpoint modulation, and cytokine signaling.



Vaccine/Cell Therapy

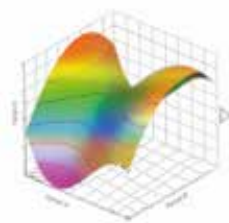
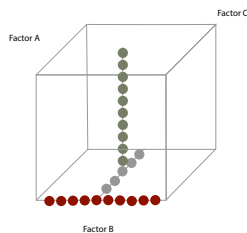
The therapy is the living cell. The Critical Quality Attributes (CQAs) of those cells including their surface marker expression, secretome, metabolome and other biophysical properties need to be defined to establish QC Release criteria.

Multiparametric Approach to Quality by Design (QbD)



Using living cells provides a more physiological response. A challenge in designing and executing cell-based assays is identifying and controlling variability. As living entities, cells sense and respond to their environment. Simple manipulations can cause them to change their properties. A poorly controlled assay can cause a manufacturing process to appear out of control or result in invalid or re-test scenarios. QbD decreases the product risk and increases process knowledge using a scientific framework.

Flow cytometry can provide multiparametric data at single-cell resolution enabling simultaneous monitoring of Critical Method Parameters (CMAs) and interrogating complex interrelated responses. Assessing cellular responses on multiple levels can lead to more efficient identification of CQAs.



One factor at a time (OFAT, panel A) versus the multifactorial Design Of Experiment (DoE, panel B) better identifies efficient design space for QbD analysis..

FLOW CYTOMETRY ASSAYS

At Beckman Coulter Life Sciences, our flow cytometry division has been developing flow cytometry assays for highly regulated clinical diagnostic testing. Our knowledge of robust assay design and a focus on the workflow positions us as a strong partner for developing flow cytometry-based solutions for complex cell-based assays. Mitigating sources of variability begins during assay design, continues during analytical method development, and through process monitoring.

We are committed to providing tools to improve the quality of data used to make critical decisions in process development through batch release testing.

- Reduce labor intensive administrative tasks
- Reduce pipetting errors and reagent inventory management
- Facilitate compliance with quality system and regulatory requirements
- Reduce subjectivity in data analysis and manage data securely

Improve confidence in data while reducing administrative burden and costs

Variety of Solutions to Help Address Current Challenges



Flow cytometry Assays by Development Stage and Regulatory Framework

Drug Discovery	Non-clinical Testing	Clinical Testing
Non-Regulated Target identification Mechanism of action Biomarker identification	GLP Drug toxicity Pharmacodynamic (PD) biomarker Pharmacokinetics Immune monitoring Receptor occupancy analysis GMP Potency and batch lot release	IVD or CLIA Drug safety and efficacy Companion diagnostics GLP Pharmacokinetics/immunogenicity Pharmacodynamic (PD) biomarker GMP Potency and batch lot release

AUTOMATED CELLULAR ANALYSIS BY FLOW CYTOMETRY

Advances in both flow cytometry and liquid handling systems combine to make walkaway automation of an entire flow cytometry assay, including multistep sample preparation and cell staining, possible. Reproducible processing of hundreds of samples a day provides the scale required for biodiscovery and cell therapy development pipelines.

Integrated Solutions Team

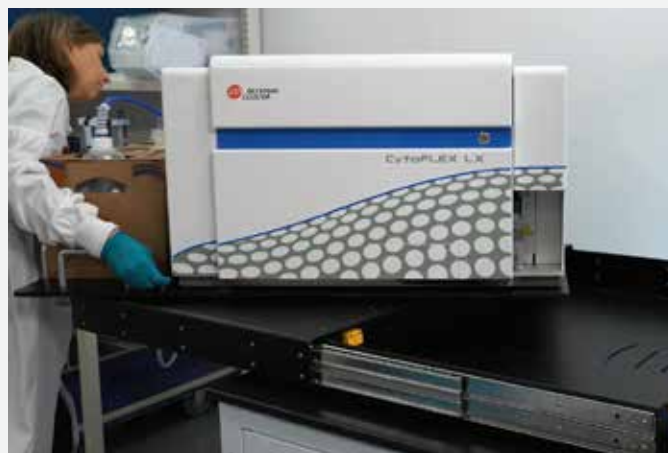


We provide a full spectrum of automation, from simple devices to complete robotic systems. Our Integrated Solutions team, with extensive, multidisciplinary training and experience in mechanical, electrical and systems engineering, software development, and project management, has successfully integrated more than 300 third-party devices from over 60 manufacturers to meet our customers' diverse automation needs. In addition, members on the team have the scientific background and knowledge to understand your applications so we can provide a solution that is right for your workflow.

Integration of CytoFLEX Flow Cytometer

Gain flexibility in your day by integrating your CytoFLEX Flow Cytometer to the Biomek i-Series Instruments for all steps involved in automated sample processing and data acquisition.

- Assay plates are transferred with the Biomek gripper directly to the CytoFLEX Flow Cytometer.
- Sample preparation data, such as sample ID, is correlated with the information collected from the flow cytometer
- Specialized table allows the CytoFLEX to be used in manual tube mode when not needed for an automated assay run.
- Onboard cleaning solutions can also be monitored and replaced as needed without dismantling the system



The table can be swiveled to allow the instrument to be used manually in tube or plate mode. Also gives easy access to onboard reagents.

BIOMEK i-SERIES

Multistep sample processing

Integrate on-deck centrifugation or plate washers for higher complexity sample preparation requirements.

HEPA Filtration

Add HEPA filtration as an optional component. Additional sample-, environmental- and/or operator-protection solutions are available.

We collaborate with third-party vendors to meet BSL-2 and BSL-3 lab needs

Shake

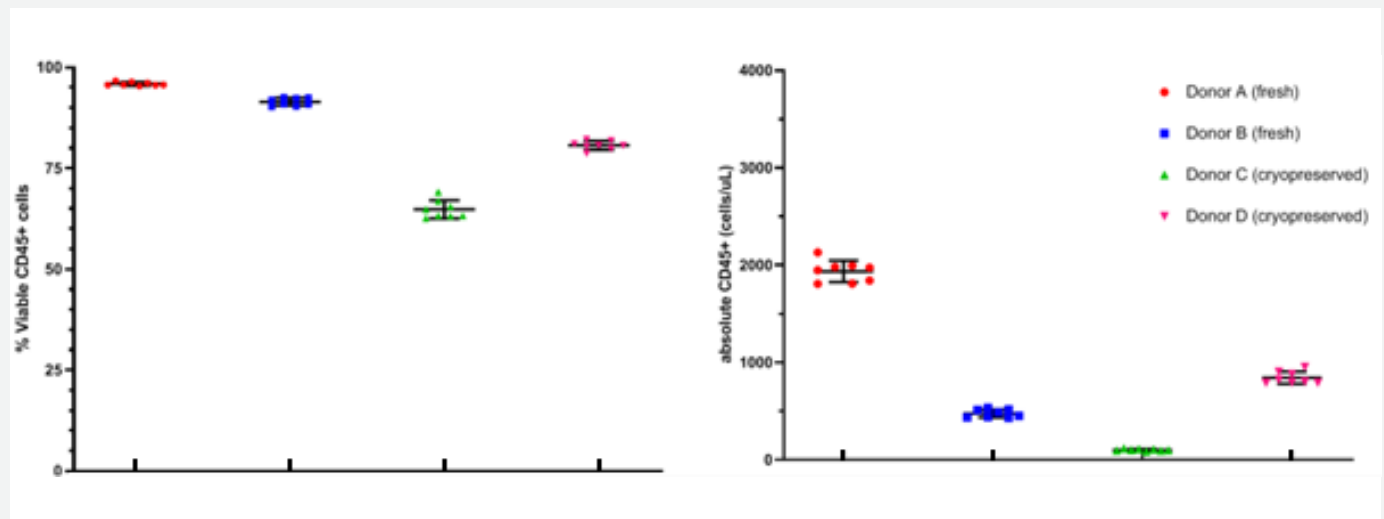
Achieve thorough mixing via a variety of on-deck devices. Easy integration of hotel/tower shakers supports the need for long-term shaking incubation.

Incubation

Include workflow solutions that meet the requirements for assay plate temperature, CO₂ and humidity environment needs.

Buffer & reagent management

Enhanced functionality enables easy access to standard on-deck reservoirs. Just-in-time reagent-filling reservoirs enable high-throughput, on-deck volume accessibility. Temperature-controlled devices help maintain the integrity of your reagents, buffers and cells.

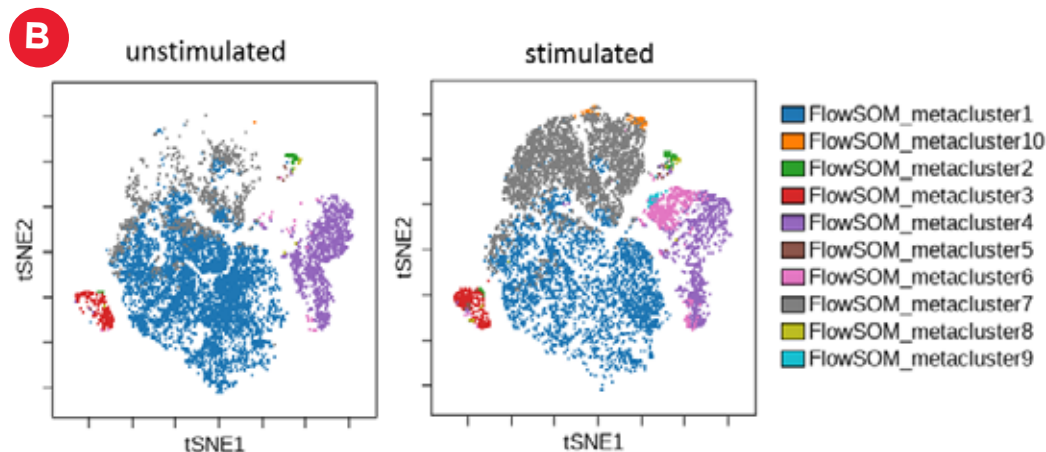
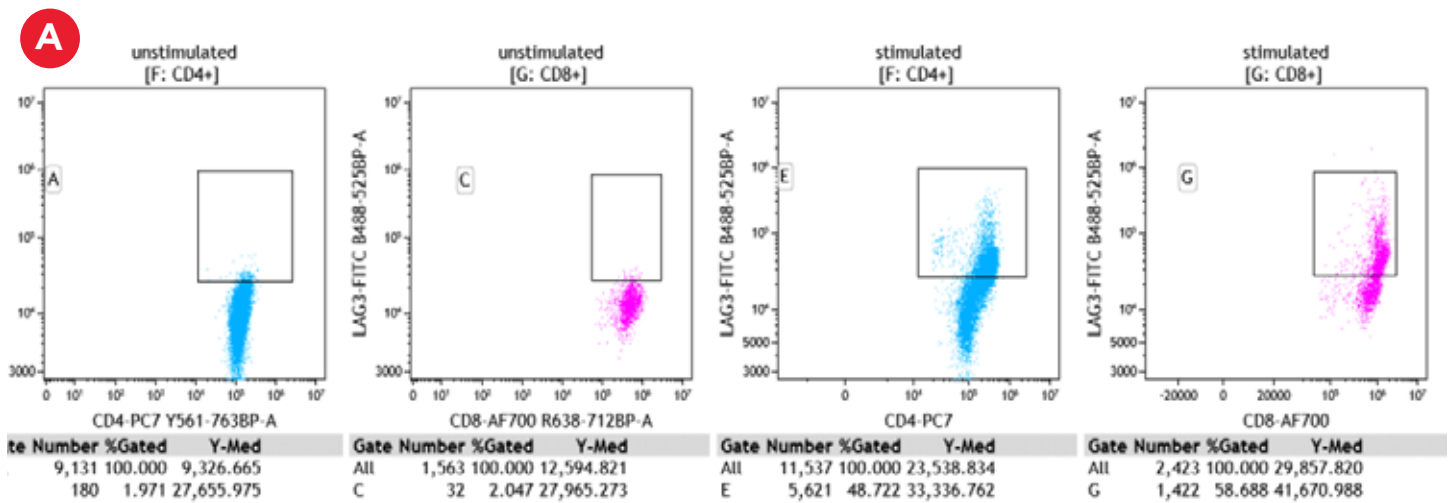


Fully Automated Viable Leukocyte Cell Counting Assay. PBMCs were isolated from blood from four healthy donors and analyzed directly or after cryopreservation, as indicated. Cell counts and percent viability was assayed (n=8) using CD45, 7-AAD, and fluorescent counting beads in a dry, unitized plate-based assay prepared in our LUCID Panel Design and Cocktailing Custom Service. The Biomek i7 Automated Workstation was used to add sample to the plate and perform all mixing, incubation, reagent additions prior to moving the plate to the CytoFLEX LX flow cytometer with plate loader. Data was acquired with CytExpert software. For each sample the Absolute counts and percent viable measurement for each replicate are displayed in a column scatter graph with lines indicating the mean and standard deviation of each set.

CytoFLEX Flow Cytometry Platform

The CytoFLEX Platform is a compact system with innovative technology borrowed from the telecommunications industry that optimizes excitation and emission, minimizing light loss and maximizing sensitivity. The same CytExpert software is used for acquisition and analysis across the entire platform. With 88% of surveyed organizations agreeing that the CytoFLEX was easy for lab personnel to learn and master.

- Exquisite sensitivity
- Small particle analysis in a benchtop analyzer
- Extensive set of repositionable band pass filters
- Flexibility to upgrade by adding additional parameters
- Intuitive software to facilitate multicolor analysis
- When installed in Electronic Management mode, 21 CFR Part 11 compliance tools are enabled.



Analysis of Stimulated and Unstimulated PBMCs T cells. PBMCs were isolated from blood from a healthy donor and cultured for 4 days either with (stimulated) or without (unstimulated) anti-CD3 and anti-CD28 coated beads prior to cryopreservation. Cultured cells were then stained using a dry, unitized deep-well plate-based assay prepared in our LUCID Panel Design and Cocktailing Custom Service consisting of backbone markers CD45-KrO, CD3-APC-A750, CD4-PC7, and CD8-A700. Additional markers for activation state were added as liquid add-ins, including CD25-PE, LAG-3-FITC, TIM-3-APC and PD-1-PC5.5 as well as ViaKrome 808 Fixable Viability Dye. Data was acquired using CytExpert software and analyzed using Kaluza Analysis Software. LAG-3 expression measured from unstimulated (blue dot plot) or stimulated (pink dot plot) CD4+ and CD8+ populations as indicated are displayed (panel A). Panel B shows the same wells analyzed with FlowSOM using the Cytobank Platform (panel B).

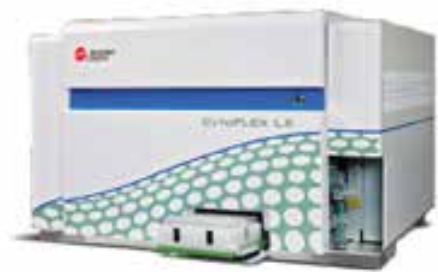
*TechValidate Research on CytoFLEX, <https://www.techvalidate.com/product-research/beckman-coulter-cytoflex> Accessed 3/25/2020.



Up to 3 lasers



Up to 4 lasers



Up to 6 lasers

CytExpert Software Features for Plate-Based Acquisition and Analysis

Setup

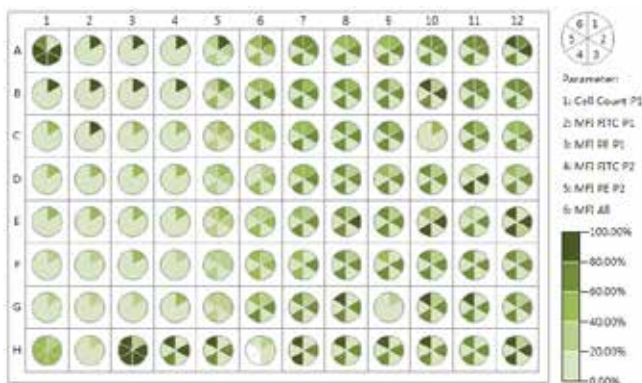
- Upload a CSV file with metadata for each well
- Ten fields available for sample identification
- Sample groups are recognized and color coded

Acquisition

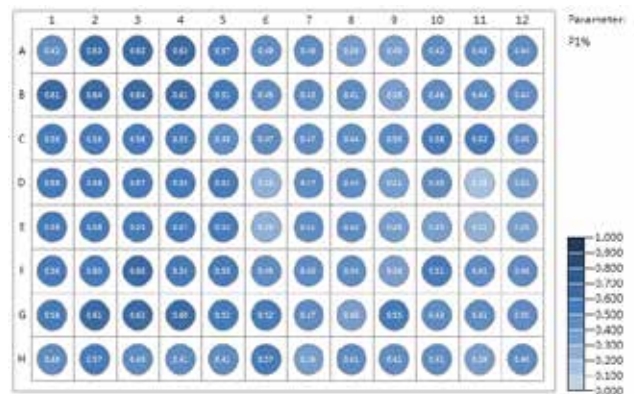
- Use the plate protocol to create groups of wells
- Control acquisition settings for each group
- Set the Mix/backflush and stopping rules for each well

Analysis

- Values can be displayed on single parameter heatmaps
- Heatmaps with up to six parameters are available
- Use custom expressions to display statistics and calculations



Color encoded value in multiparameter heatmap



Single parameter heatmap displaying value

CytoFLEX Automation Assistant

The Automation Assistant for the CytoFLEX Platform increases sample throughput by automating the loading of plates into the instrument.

- Accommodates multiple instruments, ambient plate hotels and automated incubators to scale to your workflow needs
- Includes bypass function to reprioritize the order specific plates are acquired
- Remote control communication allows efficient process coordination between the cytometer and the robotic plate loader



Dried Antibody Reagents

Our proprietary dried reagent manufacturing process, powered by DURA Innovations technology, results in a room temperature stable reagent. While unitizing the assay replaces extensive management of single-color reagent inventory, the drying takes lab efficiency to the next level by allowing storage at room temperature for worry free storage and shipping. Dry reagent technology protects the reagent, maintaining performance over time.

DURAClone Antibody Panels¹

Dry pre-formulated antibody panels for rare event detection, immune function, immune system research.

- Proven marker combination supported by peer reviewed citations
- Open channels to add drop-in markers to modify for different research questions
- Designed for assay precision and lot-to-lot reproducibility
- Includes lot matched tandem dyes for compensation controls for improved accuracy
- Includes optimized sample preparation and staining procedure
- Streamlined workflow, just add sample, incubate, wash and acquire

Visit beckman.com/reagents/coulter-flow-cytometry/antibodies-and-kits/duraclone-panels to see the complete offering



Your Clinical Research Trial Companion

DURAClone antibody panels, warranted for 12 months stability and which can be shipped and stored at room temperature, enable the level of experimental rigor that is key to multi-centric and longitudinal clinical research studies

LUCID Custom Panel Design & Cocktail Services

This service offers flexibility to design a reagent & panel configuration, using Beckman Coulter's large antibody portfolio, as well as choose the preferred reagent format in order to simplify workflow and minimize time spent on manual processes.

Dry Reagents

Dry (not lyophilized) custom reagents using the DURA Innovations technology
Available in tube or microplate format as reagent reservoir
Optimized, multi-conjugate combinations
Stable at room temperature (18°C - 30°C)
Shelf life warranted for 18 months
Includes Universal Compensation Kit

Liquid Reagents

Single-color conjugations
Optimized, multi-conjugate combinations
Bulk purified antibodies (you specify concentration and buffer)

Visit beckman.com/reagents/coulter-flow-cytometry/custom-services/custom-design-services to learn more about the ordering process













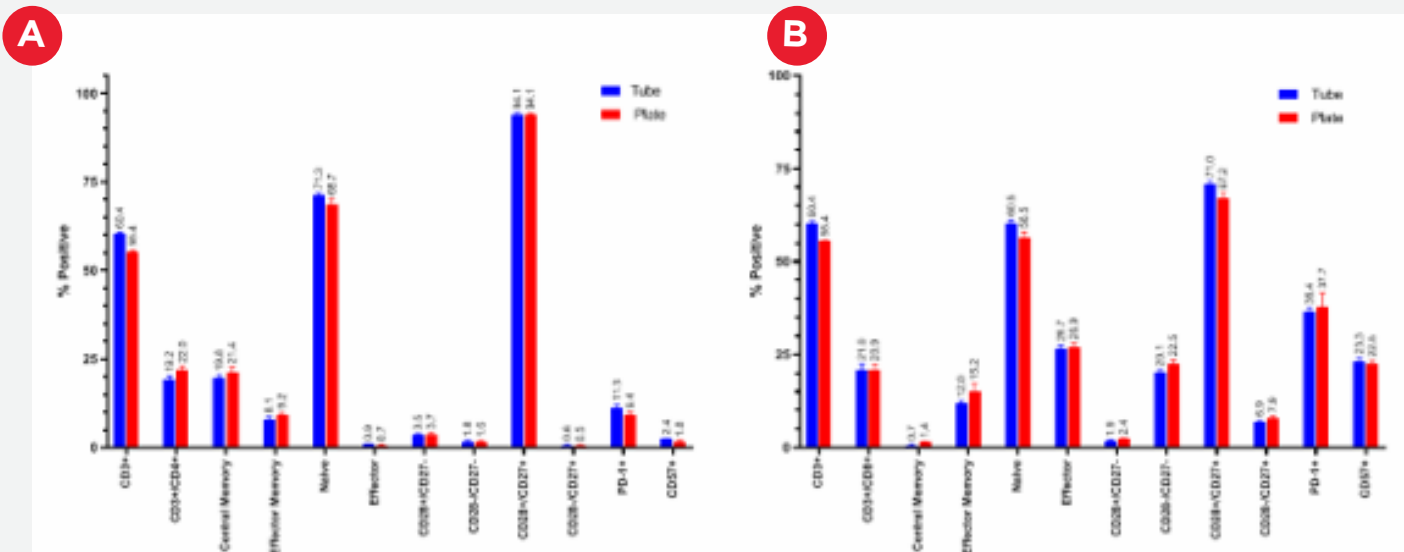
Beckman Coulter Life Sciences' Flow Cytometry Antibody Portfolio

Panel Design & Workflow

Standards, Control, Stability

Flexibility, Creativity, Change

	Dry Reagents				Liquid Reagents	
Product Type	Off-the-shelf	Custom	Off-the-shelf	Custom		
Product Line	 ClearLLab Panels	 RESOURCE	 DURAClone and DURActive	 LUCID	 Multi Color Cocktails	 Single Color Antibodies
Descriptions	IVD L&L* ² immunopheno-typing panels	Contract Manufacturing Services (CMS) ³	Dry antibody panels and stimulation mixes for clinical research ¹	Custom Panel Design & Cocktail Services (CDS)	Liquid, pre-mixed antibody cocktails	CE-IVD, ASR and RUO conjugated antibodies
Technology						

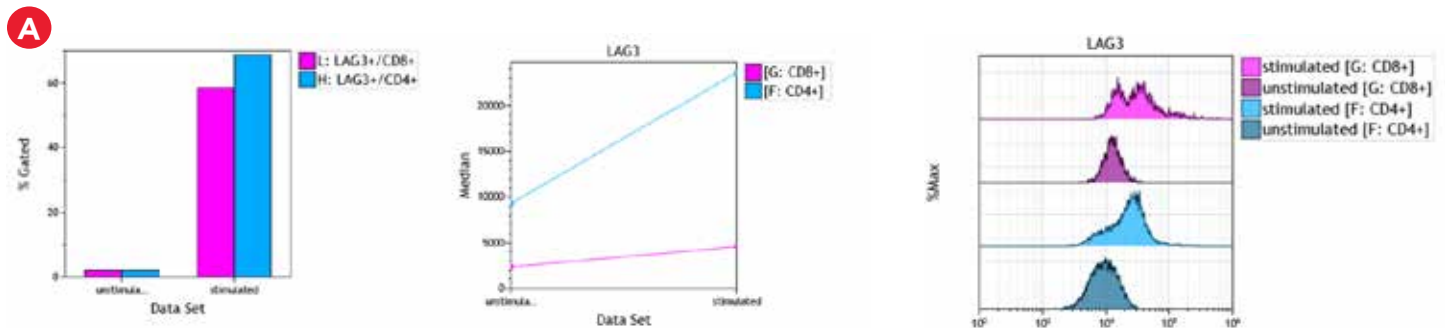


Comparison of DURAClone and Custom Dry Plates Performance. PBMCs from a healthy donor were stained with DURAClone IM T Cell (Part number B53328) or with a dry, unitized plate-based assay prepared in our LUCID Panel Design and Cocktailing Custom Service. Panels contained the same reagents but were not lot matched. Data was acquired on a CytoFLEX LX U-V-B-Y-R-I flow cytometer and analyzed in Kaluza Analysis software following DURAClone IM T Cell instructions for use. The bar graphs compare the population percentages for each subset analyzed and error bars represent SD across 6 replicates. Panel A is CD4+ subsets and Panel B shows CD8+ subsets

Kaluza Analysis Software

Kaluza Analysis Flow Cytometry Software is designed to simply and efficiently analyze multicolor data. Four persistent control panels provide access to every aspect of the data. Any changes made are automatically reflected in the analysis. The statistics are updated immediately and the population colors change, giving the operator real time feedback.

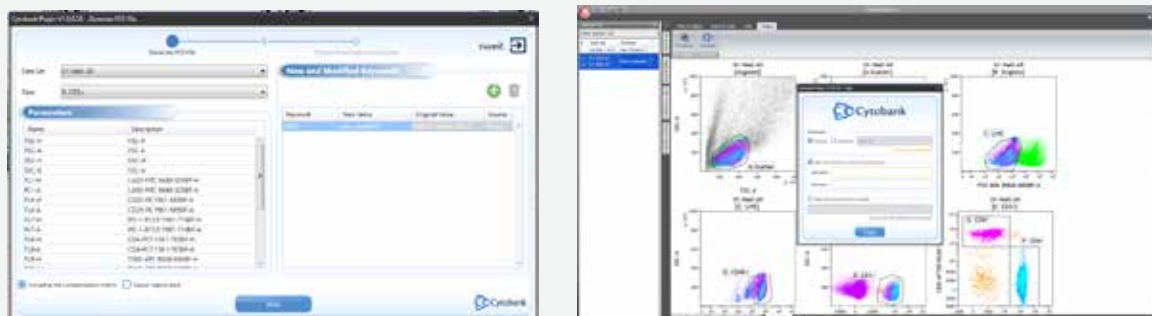
- Loads any FCS compliant file through standard 3.1
- Real time processing of multi-color files of up to 20 million events
- Real time adjustments to data display, including unlimited undo
- Context specific radial menus put functions at your fingertip
- Enhanced color compensation algorithms
- Built in batch processing for high throughput labs



LAG3 Expression Analysis. FCS files from representative wells from anti-CD3 and anti-CD28 stimulated and unstimulated PBMCs were exported and uploaded into Kaluza Analysis software. Using the composite function, three visualizations are used to demonstrate different methods to highlight differences across samples, panel A. Bar and line graphs generated in Kaluza using the Comparison plot allow the visual comparison of statistical results without the need to export data to another software package. Different histogram types enhance the qualitative display of data. Panel B shows an example of a customized report, including digital signature for documenting reviews.

Cytobank Plugin for Kaluza

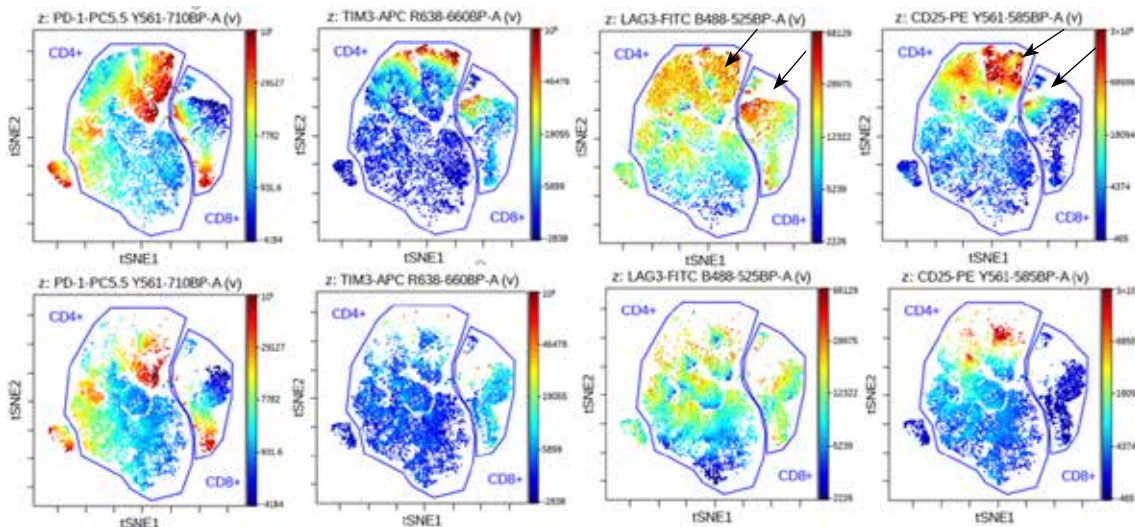
Use the powerful tools in Kaluza to prepare data for Machine Learning Analysis. Use the real time feedback and efficient interface tools to adjust compensation, transform the scaling, and provide a quick overall assessment of data integrity. The Cytobank Plugin then uploads the file to your Cytobank account for further analysis by machine learning algorithms, data sharing and archiving. Use the de-identifier function to remove any personal identifying information or other FCS keywords from clinical trial data to maintain confidentiality.



Cytobank Cloud-Based Platform

Quickly summarizing high parameter data in intelligible, comprehensive figures can increase productivity. The compelling graphics offered by the platform enable unparalleled communication between clinical researchers, scientists and other collaboration partners.

- Analyze multiparameter cytometry data
- Visualize findings and produce high-impact graphics
- Archive data for ready access and compliance
- Collaborate with colleagues across disciplines and geographies



Activation Marker Expression Levels Using viSNE Algorithm. FCS files from representative wells from anti-CD3 and anti-CD28 stimulated and unstimulated PBMCs were uploaded into the Cytobank platform and dimensionality reduction using viSNE was conducted. Expression levels for each of the T cell activation markers is shown in color for both stimulated (top row) and unstimulated (bottom row) samples. CD4+ and CD8+ subsets were gated on the viSNE maps. Activated cells cluster at the top of the viSNE map (arrows) and the upregulation of the investigated markers is shown across all events.

Cytobank Data Management and Collaboration

Cytobank platform's cloud-based capabilities allow scientists from around the globe to collaborate and dive deeper into large and complex data sets. Share and access data anytime, anywhere from any web-enabled device.



The Experiment Manager allows you to search your own experiments as well as experiments that have been shared with you. By organizing experiments in projects you can assign different levels of access. The Tree view clearly shows relationships between experiments and makes it easy to navigate to the advanced analysis you are looking for. In addition to preserving your cytometry experiments, Cytobank's secure servers allow you to back up and associate related experimental data, including protocols, presentations and microscopy images.



Choose Beckman Coulter Life Sciences for Benchmark Expertise and Innovation

For over 80 years Beckman Coulter has driven innovation. We remain committed to shaping flow cytometry technology to fit seamlessly into your lab's workflow and to provide an optimal user experience. When you choose a Beckman Coulter instrument you receive the highest level of expertise, innovation, and quality assurance.

Contact your local Beckman Coulter sales representative.

beckman.com/contact-us

1. *Does not include the IVD kits, DuraClone B27 and DuraClone Tri-T-STAT.
2. *Leukemia & Lymphoma, non-Hodgkin Lymphoma Only.
3. *For CMS/RESOURCE reagents, the customer is the legal manufacturer of the product. The customer provides the formulation of the reagent combination and bears responsibility for any legal or regulatory requirements for the finished product including validation, product label content, intended use claims and regulatory approvals or clearances based on the product's final use.



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