Merck Millipore
Bioscience Instruments








Cellular Analysis

Amnis® Imaging Flow Cytometers
guava® easyCyte™ Flow Cytometers
Muse™ Cell Analyzer
Scepter™ 2.0 Handheld Automated Cell Counter
CellASIC™ ONIX Microfluidic Platform

Luminex® Instruments

MAGPIX®
Luminex® 200™
FLEXMAP 3D®

Spectrometry

Direct Detect™ Protein Quantitation System

Western Blotting & IHC

SNAP i.d.® 2.0 system for Western Blotting and IHC

Water Purification Systems

Milli-Q® Integral
Simplicity®
Milli-Q® Direct

Merck Millipore is a division of MERCK

Cellular Analysis

Merck Millipore's cellular analysis platforms give you instant access to all facets of cellular phenotypes. From simple and quick cell counts, multidimensional cell health assessments, and sophisticated multiparameter measurements on individual cells, Merck Millipore's cell analysis equipment deliver more predictive and reliable data compared to other cell analysis instrument to enhance your experiments. These solutions incorporate optimized kits and assays linked to intuitive analysis software, and are based on powerful proprietary technologies. Our systems make both basic and complex cell analysis simple, widely accessible, and affordable.

Learn more about how our automated, affordable, easy-to-use Cell Analysis platforms help you make faster, more accurate decisions about your experiments, allowing for more productive research. With accurate, intuitive information about your cells, Merck Millipore's cellular analysis equipment ensure your research success!

- ## Amnis® Imaging Flow Cytometers
  High Speed Quantitative Image Analysis of Cells in Flow
- ## guava® easyCyte™ Flow Cytometers
  Flexible, open flow cytometry formats from single-sample to 96-well plate analysis, and options for detecting up to 12 parameters
- ## Muse™ Cell Analyzer
  Bench space cell analysis enabling cell heath & phenotype determination in a closed system
- ## Scepter™ Cell Counter
  Handheld automated cell counting
- ## CellASIC™ ONIX Microfluidic Platform
  Perfusion-based system enabling automated changes to culture conditions


Research Area	Amnis®	guava®	Muse™	Scepter™
Viability	●	●	●	○
Apoptosis	●	●	●	
Cell Cycle	●	●	●	
Cell Signaling	●	●		
Stem Cells	●	●		
Immunology	●	●		
Cancer	●	●		
Mutation & Toxicology	●	●		
Drug Discovery	●	●		
Others	Nuclear translocation	Absolute cell counting	Absolute cell counting	Absolute cell counting
	Intracellular molecular trafficking	SpaceCraft		Absolute cell size
	Shape change	Radiation Exposure		
	Localization & Co-localization	Biofuels (Microalgae)		
	Subcellular compartmentalization	Coal to Natural Gas		

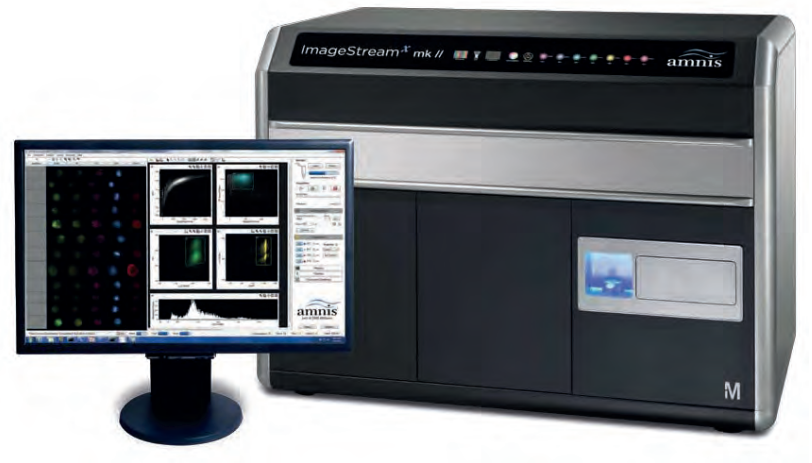
● Qualitative & Quantitative ○ Qualitative

Amnis® Imaging Flow Cytometers

ImageStream^{X} Mark II
FlowSight®

Integrating flow cytometry and microscopy to advance discovery. Amnis builds two capable and versatile lines of cell analyzers to meet a wide range of flow cytometry and cell imaging applications.

- ImageStream^{X} Mark II; only instrument combining statistical power of flow cytometry with the insights of high-resolution microscopy of every cell
- FlowSight® ; exceptional flow capabilities with visual confirmation provided by imagery of every cell
- Multiparameter flow cytometry via up to 12 channels plus quantitative & visual location of that fluorescence within the cell
- Innovative, capable & versatile; both platforms are upgradable & can be adapted to meet current & future research needs



Technology

Integrating flow cytometry and microscopy to advance discovery. Amnis builds two capable and versatile lines of cell analyzers to meet a wide range of flow cytometry and cell imaging applications.

Flow Cytometry

Fast, sensitive and quantitative multispectral probe analysis on large populations of cells

Spatial resolution - No location information

Fluorescence Microscopy

Good spatial resolution

Small cell populations, Low throughput,
No spectral compensation,
No side scatter image,
Difficult to find rare subsets of cells

Amnis Imaging Flow Cytometer

Fast, sensitive and quantitative multispectral probe analysis on large cell populations

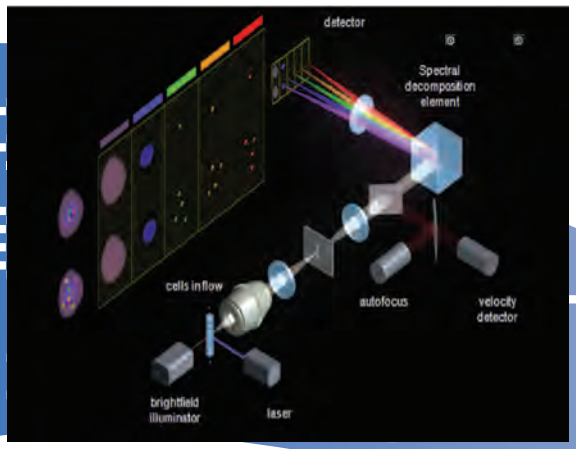
Spatial localization of probes on, within & between cells possible

Enables statistically robust microscopic cell analysis

Comparison of the Imaging Flow cytometer and traditional Flow cytometer & Microscopy

	Flow Cytometry	Microscopy	Imaging Flow Cytometer
High speed	+	-	+
Statistical power	+	-	+
Imaging	-	+	+
Information content	-	+	+
Research benefit	Objective, statistical discrimination of cells based on intensity	Discrimination of cells based on appearance	Objective, statistical discrimination of cells based on appearance

Imaging Flow Cytometer's Architecture



Integrating fl ow cytometry and microscopy

Choose the Instrument That's Right for You

The tables below outline the technical and application differences between FlowSight® and ImageStream^X cytometers.

Specifications	FlowSight®	ImageStream^X Mark II
Overview	Compact, high-sensitivity, multi-color flow cytometer. Produces multiple images per cell for qualitative and limited quantitative image analysis.	High-resolution microscope for suspended cells. Produces detailed brightfield, darkfield and flourescence imagery for a wide range of quantitative, statistically robust image-based assays.
Magnification	20X Fixed	60X / 40X / 20X
Pixel Area	2.0 square microns	0.1 / 0.25 / 1.0 square microns
Number of Channels	12	6 or 12 High Resolution
Max Field of View Width	64 microns	128 microns
Quantitative Image Analysis	Optional	Standard
Brightfield Illumination	2 Channels Standard	10 Channels Standard
Laser Power Doubler	Optional	Standard
375 nm Excitation Laser	X	Coming Soon
405 nm Excitation Laser	90 mW	120 mW
488 nm Excitation Laser	60 mW	200 mW Standard, 400 mW Optional
561 nm Excitation Laser	50 mW	200 mW
592 nm Excitation Laser	X	300 mW
642 nm Excitation Laser	100 mW	150 mW
730 nm Excitation Laser	X	Coming Soon
785 nm Excitation Laser	10 mW	70 mW
Sample format	microcentrifuge tube	microcentrifuge tube
AutoSampler	Optional	Optional
Extended Depth of Field	X	Optional
Sensitivity	10 MESF	5 MESF

ImageStream^X Mark II

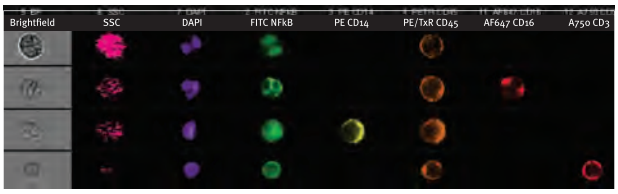
Measure more accurately and reliably

The ImageStream^X Mark II is the third generation of the ImageStream line of imaging flow cytometers and the result of over 10 years of development. The raw power of the ImageStream^X for cell analysis is unmatched: it produces up to 12 high resolution images of each cell directly in flow, at rates up to 5,000 cells per second, and with greater fluorescence sensitivity than the best conventional flow cytometers.

These breakthrough capabilities allow scientists to quantitate cellular morphology and the intensity and location of fluorescent probes on, in, or between cells - providing access to a broad range of image analysis applications, including nuclear translocation, shape change, internalization, autophagy and more.

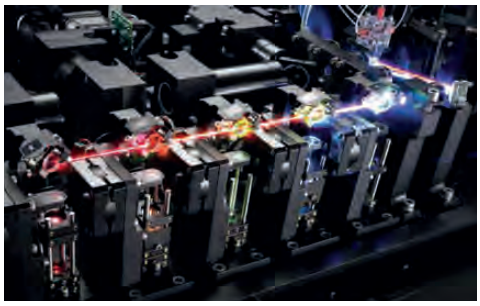
▸ Advantage

- **Faster:** The Mark II can analyze up to up to 5,000 cells/sec with real-time intensity compensation - ideal for very rare cell analyses.
- **Easier:** The all-new and highly intuitive user interface provides real-time plotting and graphical gating, as well as images of every cell. The easy-to-use compensation wizard quickly guides you through the setup of multi-color compensation matrices.
- **More flexible:** The Mark II accepts up to 7 lasers and works with sample volumes of 20-200 ul for added experimental flexibility - perfect for multi-user laboratories.
- **More efficient:** Improved fluidics provide up to 95% sample utilization for high yields with rare cell samples. Unused sample can be recovered for further analysis.
- **More affordable:** The Mark II is even more affordable with two excitation lasers, data analysis software.



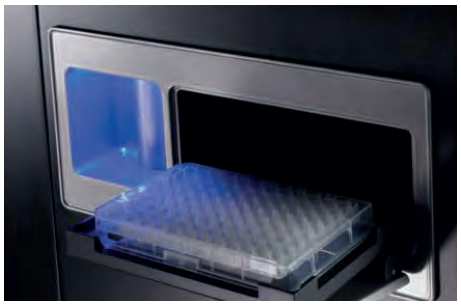
12 Image Channels

The 12 Image Channel option greatly expands the experimental flexibility and analytical potential and of the ImageStream^X by doubling the number of images per cell from six to twelve.



7 Excitation Lasers

The standard 488 nm laser of the ImageStream^X Mark II may be augmented with up to six additional lasers, including 375, 405, 561, 592, and 642 nm.
A high-power 488 nm laser upgrade is also available for even higher sensitivity detection of 488 nm excited fluorescent dyes.



AutoSampler for Multiwell Plates

The AutoSampler option for the ImageStream^X Mark II imaging flow cytometer enhances productivity with unattended sample loading from multiwell plates. The fully integrated AutoSampler option allows you to easily perform dose response and time course studies even in primary samples, thanks to the ImageStreamis ability to image large numbers of cells from each sample.

** Additional module - MultiMag and EDF (Extended Depth of Field)

■ FlowSight®

Flow cytometry with vision

FlowSight® is a compact 12-channel flow cytometer that provides high-end performance and images every cell. FlowSight® can accommodate four lasers, an AutoSampler, and a Quantitative Imaging upgrade to suit beginners and experts alike. Despite its incredible capabilities, the FlowSight® is surprisingly affordable.

▸ Advantage

- **Capable: Sensitive and flexible for every need**

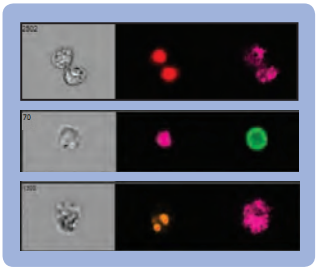
The FlowSight® offers high performance in a small package. Its innovative design increases signal and minimizes noise to provide unmatched fluorescence sensitivity. Twelve standard detection channels simultaneously produce brightfield, darkfield and up to ten channels of fluorescence imagery of every cell. With these unique capabilities, the FlowSight® enables a broad range of applications.

Twelve channel imagery of 3 micron diameter Spherotech 8-peak Rainbow beads

- **Intuitive: Easy-to-use, with imagery for every cell**

The FlowSight® operates like a conventional flow cytometer but also provides imagery of every cell. Powerful and Intuitive analysis software seamlessly links quantitative data to imagery:

- Click on a dot in any plot to see the corresponding cell imagery.
- Click on a bin in any histogram to view all the cells in that bin.
- Draw gates on dot plots and view the resulting populations to validate results.

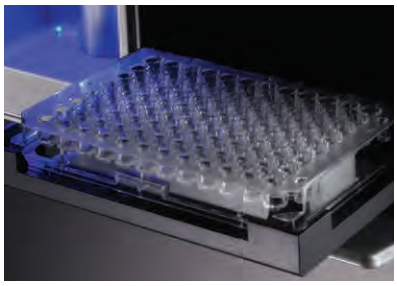


- **Affordable: Designed and priced for every lab**

The FlowSight® is powerful enough for the core lab but sized and priced for any lab. The system can be factory configured or field upgraded with up to four excitation lasers (405, 488, 561, 642 nm), a 96-well plate AutoSampler, and a powerful quantitative image processing option. Whether in a base configuration or fully-optioned, the FlowSight® sets a new standard of value.

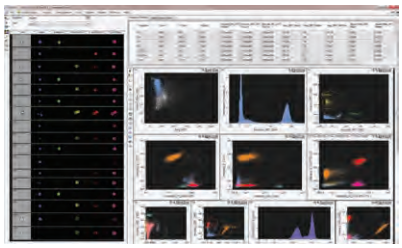
Excitation Lasers

The standard 488 nm blue laser of the FlowSight® system may be augmented with up to three addtional lasers at 405 nm (violet), 561 nm (green), and 642 nm (red) wavelengths. Adding excitation lasers increases experimental flexibility by permitting a broader palette of fluorescent markers. All lasers are intensity adjustable to ease protocol development.



96 well AutoSampler

The AutoSampler option for the FlowSight® enhances productivity with unattended sample loading from 96 well plates. The fully integrated AutoSampler option greatly facilitates dose-response and time-course studies.



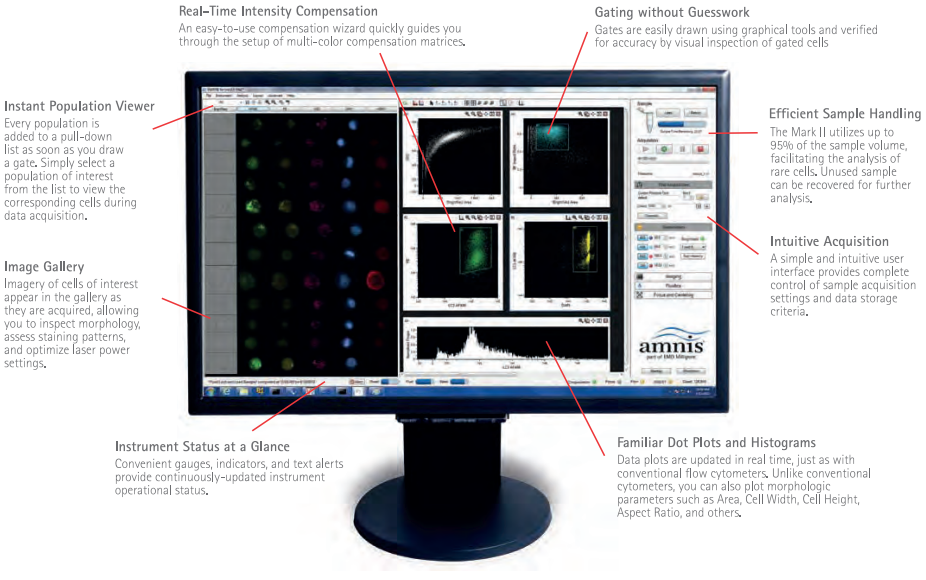
Quantitative Imaging upgrade

The Quantitative Imaging upgrade includes optical, computer, and software enhancements that increase the performance of the FlowSight®. Improved optics yield better image quality, better sensitivity and more brightfield options. Optical improvements include increased resolution (1.0 um^2 vs. 2 um^2 pixel area) and a laser power doubler to increase sensitivity. The post-acquisition image processing software allows highly quantitative determinations of the location and strength of fluorescence signals for applications such as nuclear translocation and shape change.

Software

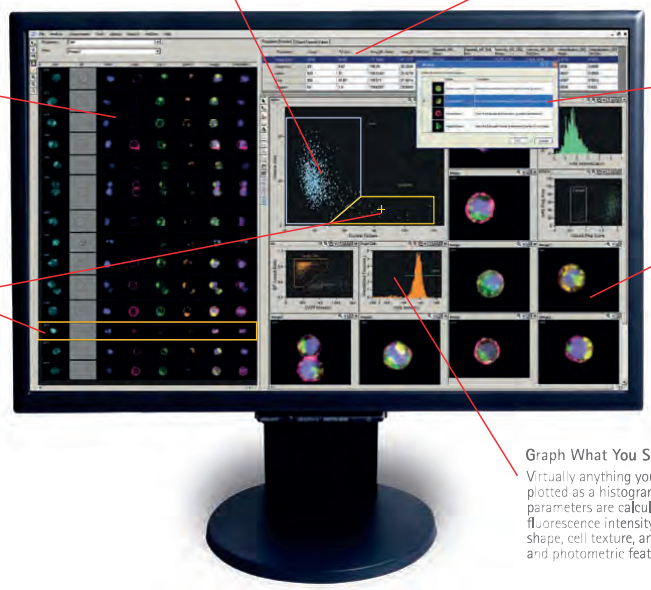
INSPIRE

The INSPIRE acquisition software developed for the Mark II provides a dramatically simplified user interface, much closer in look and feel to that of the FlowSight® and conventional flow cytometers. As in previous ImageStream instruments, the user will have the ability to see cell images as they are acquired. However, this capability will be expanded to include the visualization of specific sub-populations that are graphically selected by the user. Once a gate is drawn, the population will appear in a pull- down list for display in the image gallery.



IDEAS

IDEAS analysis software includes wizards for popular applications. "Best Feature Finder" wizard compares Positive and negative control results and automatically determines highest scoring feature for any analysis.



Graphical Population Definitions
Define populations using familiar graphical tools and combine them with logical functions.

Comprehensive Population Statistics
Characterize your cell populations with a wide range of statistical metrics to reveal differences in cell morphology, phenotype, and function.

Inspect Your Populations
The Image Gallery allows you to see every image of every cell or perform a "virtual cell sort" to inspect and validate the cells within a specific population.

Wizards Simplify Analysis
Pre-configured and optimized analysis wizards are provided for many common applications.

Flexible Image Display Tools
Create composite images, pseudo-color representations and a host of other image transformations for reporting and publication.

Images for Every Dot
Every dot in every scatter plot is linked to the corresponding cell imagery. Simply click on a dot to see the associated cell images or vice-versa.

Graph What You See
Virtually anything you see in the imagery can be plotted as a histogram or dot plot. Hundreds of parameters are calculated for every cell, including fluorescence intensity, fluorescence location, cell shape, cell texture, and numerous other morphologic and photometric features.

Applications

A Wealth of Applications

Shape Change, Autophagy, Cell Signaling, Trafficking, and more

Quantitative imaging – not just observations

Microscopy offers detailed cellular images and morphologic information, which are useful scientific tools for the study of cell function. However, the interpretation of microscopic imagery can be subjective, qualitative, and laborious.

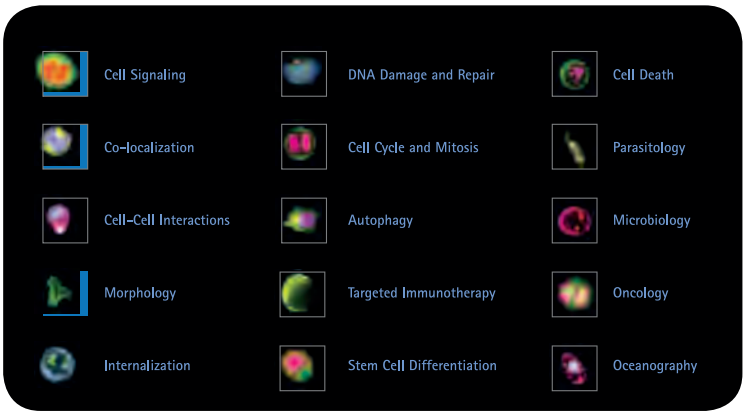
Flow cytometry is excellent for quantitative phenotyping and yields statistically robust results by rapidly interrogating large numbers of cells. However, flow cytometry lacks any ability to image, so sub-cellular localization and cell function are measured indirectly.

By combining the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insight of microscopy, the ImageStream^X Mark II overcomes the limitations of both techniques and opens the door to an extensive range of novel applications.

Any application you can imagine

The ImageStream^X Mark II is designed to be a general-purpose platform for cellular studies and is not limited to the applications illustrated in this brochure.

The ImageStream^X Mark II utilizes the same dyes and markers employed in microscopy and flow cytometry and can perform virtually any standard flow cytometry assay with the added value of visual confirmation.

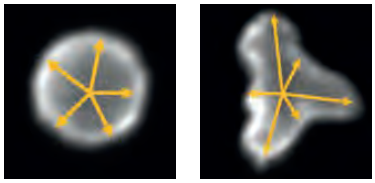


Featured applications

The applications detailed on the following pages demonstrate the types of studies that can be performed using the ImageStream^X Mark II and its powerful companion IDEAS image analysis software. Over 250 peer-reviewed publications incorporate ImageStream studies.

Morphology

Change in cell shape is correlated with change in function, particularly in the case of macrophage activation, stem cell differentiation, and cellular response to drugs.
The ImageStream^X Mark II measures cell shape using powerful, pre-defined features in the IDEAS image analysis software. One such feature is the Circularity score:



The Circularity score is a measure of how much t he cell radius varies. Round cells (left) have high Circularity scores while irregularly shaped cells (right) have low Circularity scores.

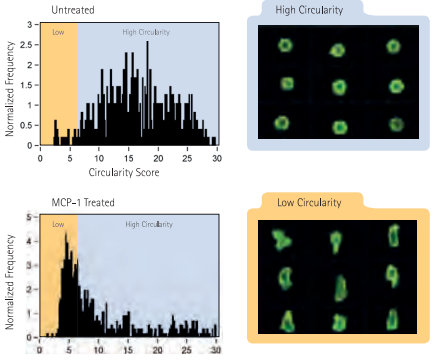
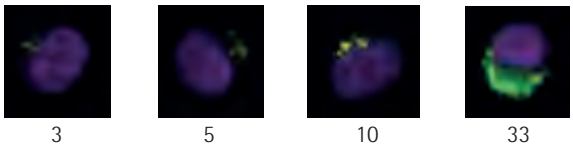


Figure 1. Shape Change in Primary Monocytes
Chemoattractant MCP-1 induces monocyte shape change and migration to sites of inflammation, as evidenced by the significant decrease in the Circularity score of the MCP-1 treated sample relative to the untreated control. In contrast, treatments that reduce inflammatory response - such as drugs for autoimmune disorders - result in an increase in Circularity scores.

Autophagy

During autophagy, cytoplasmic LC3 is processed and recruited to the outer membrane of autophagosomes. Cells undergoing autophagy can be identified by visualizing LC3 puncta and enumerating the spots within each cell using the Spot Count feature of the IDEAS software package:



The IDEAS image processing software included with the ImageStream^X Mark II determines the Spot Count of every cell. In this example, cells with varying number of LC3-FITC (green) spots are shown with their corresponding Spot Count.
The experiment is described at right.

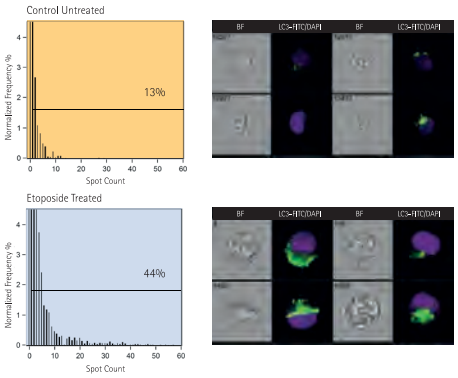


Figure 2. Autophagy in the Human CML Cell Line K562
K562 cells were treated with etoposide to induce autophagy. Representative brightfield and merged LC3-FITC (green) and DAPI (purple) images are shown above for control and treated cells. The number of LC3 puncta were quantified for each cell using the Spot Count feature of IDEAS and each sample of over 10,000 cells was characterized by a Spot Count histogram. The percentage of cells exhibiting one or more puncta increased from 13% (control) to 44% (treated).

Cell Signaling

Molecular translocation of transcription factors from the cytoplasm to the nucleus is a pivotal event in many processes critical to cellular activation, differentiation, and host defense. The IDEAS software package quantifies nuclear translocation events by automatically correlating the images of the transcription factor and the nucleus using the Similarity score.

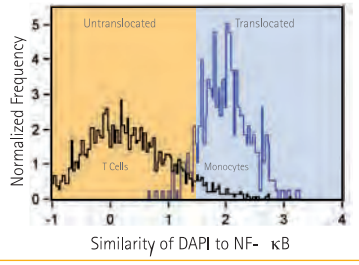
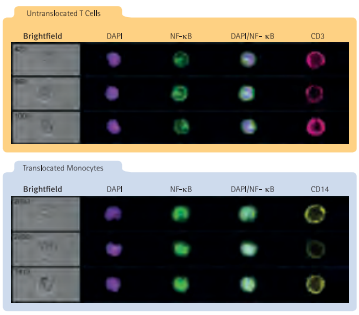


Figure 3.
Translocation of NF-kB in Whole Blood Leukocytes
NF-kB translocation is quantified in immunophenotypically-defined whole blood leukocytes imaged at 60X magnification. This example shows that lipopolysaccharide specifically induces NF-kB nuclear translocation in monocytes (blue histogram, images at lower right) but not T cells (black histogram, images at upper right).



Co- localization and Trafficking

The ImageStream^X Mark II greatly improves colocalization studies by combining the rapid collection of large numbers of cell images with objective measurement of the Similarity of bright image details.

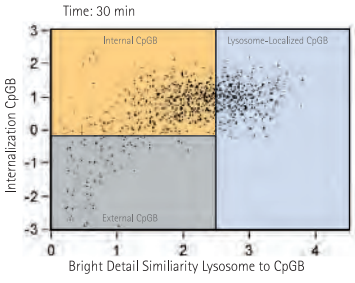
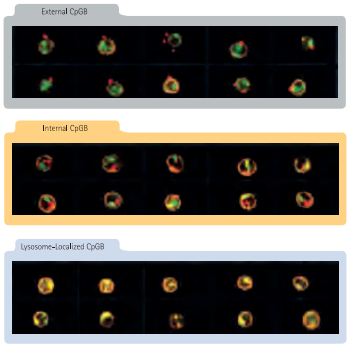


Figure 4. Internalization and Trafficking of CpGB
Lysosomal trafficking of CpGB within pDC is quantified using the Internalization (Y-axis) and the Bright Detail Similarity (X-axis) scores, and representative merged images of pDC (orange), CpGB (red), and lysosomes (green) are shown at right. Cells within the lower left region of the plot have surface-bound CpGB. As CpGB molecules enter the pDC, the Internalization score increases (upper left region). Once the CpGB traffics to the lysosomes, the similarity between the CpGB and lysosome image pair increases (upper right region). Data courtesy of Dr. Patricia Fitzgerald-Bocarsly, University of Medicine and Dentistry, New Jersey.



Flow Cytometry
guava® easyCyte™ Systems

The guava easyCyte™ flow cytometry systems are easy to use and deliver complete and comprehensive cell analysis—right on your benchtop. The culmination of over a decade of flow cytometry expertise, these instruments consume less sample, generate less waste and are easier to use and maintain than traditional flow cytometers—all while providing the analytical power you need to expand your research horizons.

Single blue (488 nm), dual blue and red (642 nm), or triple blue, red, and violet (405 nm) excitation lasers provide up to 12 simultaneous detection parameters, including 10 fluorescent colors plus forward and side scatter for size and granularity determination. The guava easyCyte™ family also meets your sample throughput needs by offering both single sample and multi-sample processing. The guava easyCyte™ HT instruments provide high throughput analysis with a robotic sample tray that automatically handles a 96-well microplate and up to 10 sample tubes, while the guava easyCyte™ systems enable single sample processing with additional cost savings.

- **Flexible**
  - Up to 12 detection parameters
  - Multi-sample or single sample processing

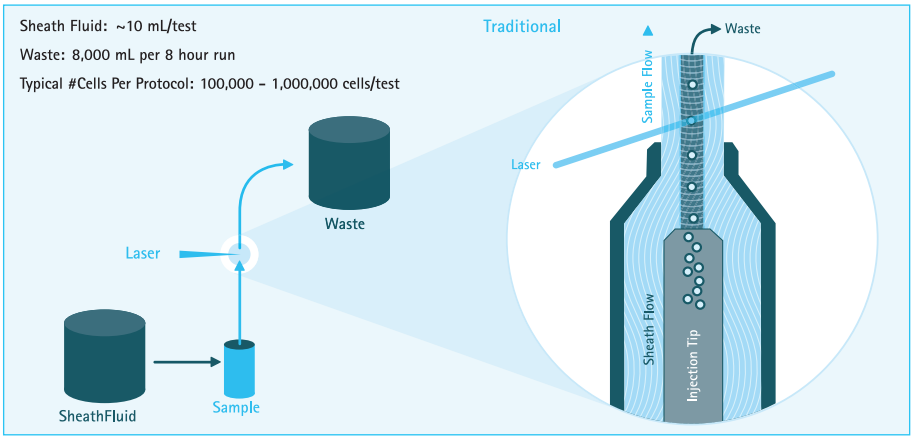
- **Easy to Use**
  - No sheath fluid required, enabling small samples and low waste
  - Intuitive software
  - Absolute cell counts-determine accurate cell numbers and populations without reference beads
  - Flow cell is self-aligning and user-replaceable for easy cleaning and maintenance.

- **Affordable**
  - Designed and priced for every laboratory and budget

Traditional Flow Cytometry

Flow cytometry is the rapid measurement of particles (cells) flowing in a stream. Since the development of the first fluorescence based flow cytometer in 1968, flow cytometry has become a major tool for cell biology research. Modern instruments are able to analyze several thousand particles every second in real time, and can actively separate and isolate particles with specified properties.

Traditional Sheath Fluid System



Principle of flow cytometry

A beam of light (usually laser light) of a single wavelength is directed onto a hydro-dynamically focused stream of fluid.
A number of detectors are aimed at the point where the stream passes through the light beam, one in line with the light beam (forward scatter or FSC), and several perpendicular to it (side scatter or SSC), and one or more fluorescent detectors. Each suspended particle from 0.2 to 150 µm that passes through the beam scatters the light in some way. Fluorescent chemicals found in the particle or attached to it may be excited into emitting light at a higher wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors.

By analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak) it is then possible to derive various types of information about the physical and chemical structure of each individual particle. FSC correlates with the cell volume and SSC depends on the inner complexity of the particle (i.e., shape of the nucleus, the amount and type of cytoplasmic granules, or the membrane roughness). Some flow cytometers on the market have eliminated the need for fluorescence and use only light scatter for measurement. Other flow cytometers form images of each cell's fluorescence, scattered light and transmitted light.

Flow cytometers

Modern flow cytometers are able to analyze several thousand particles every second, in "real time." A flow cytometer is similar to a microscope, except that instead of producing an image of the cell, flow cytometry offers high-throughput, automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

Microcapillary Flow Cytometry

The technology behind it all

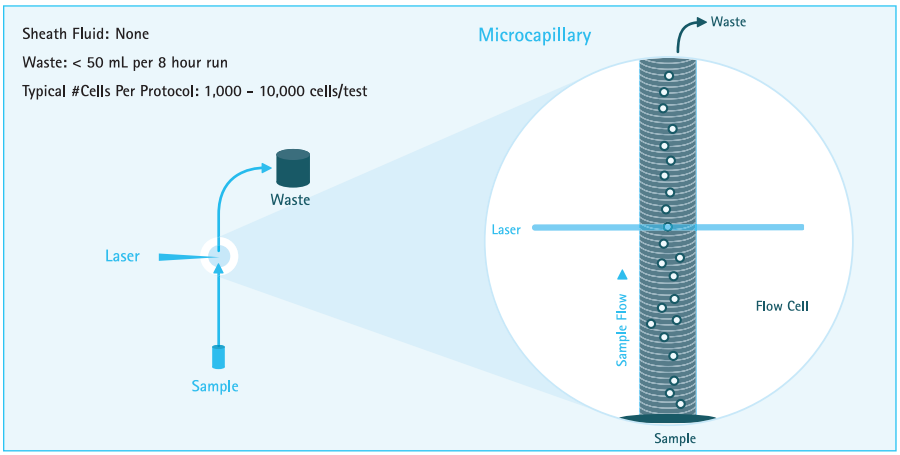
At the heart of every guava system is a unique, microcapillary flow cell that eliminates the need for sheath fluid. This translates into smaller samples, less reagents, and minimal waste, saving you both time and money. Plus, since the flow cell is self-aligning and user-replaceable, you can remove it yourself at any time for cleaning and maintenance — no more expense or downtime for service visits. And, by eliminating complicated fluidics, we've created a tiny instrument footprint that fits into the tightest spots, saving valuable laboratory space.

- No laser alignment or sheath fluid required
- Uses smaller sample volumes and generates less waste than traditional systems
- Flow cell is user replaceable for minimal downtime
- High-pressure purge available for removing obstructions in flow cell
- Aspirates sample directly from tube

Guava Patented Microcapillary Advantages

- Absolute counts
- 1/100x less sample
- 1/10-1/100x less reagents
- 1/1,000x less waste
- 1/10x less training
- 1/10x less footprint
- Less maintenance

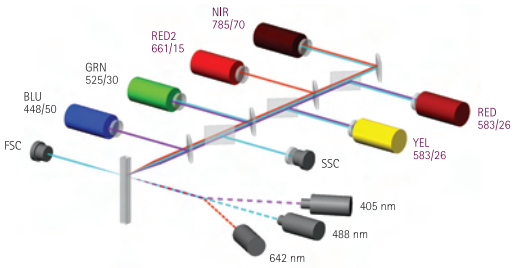
Guava-Patented Microcapillary System



Inside the guava easyCyte systems

The guava easyCyte HT flow cytometry systems are uncomplicated instruments that deliver complex cell analysis— right on your benchtop. One (blue-488 nm), two (blue-488nm and red-642 nm) or three (blue-488 nm, red-642 nm and violet-405 nm) excitation lasers provide up to 12 simultaneous detection parameters, including ten fluorescent colors plus, forward and side scatter for size and complexity determination. In the two and three-laser systems, the lasers overlap spatially and are modulated out of phase at high frequency so that each particle is sampled many times as it travels through the overlapped beams. Modulation is particularly important for identifying dyes which have overlapping emissions, such PE-Cy7 (blue laser excitation) and APC-Cy7 (red laser excitation). Unlike spatially separated beams, modulation eliminates the need for time-delay calibration, simplifying the overall operation of the instrument. The guava easyCyte™ HT instruments make possible high throughput analysis with a robotic sample tray that automatically handles a 96-well microplate and up to 10 sample tubes, while the guava easyCyte™ systems enable single sample processing with additional cost savings.

Inside the guava easyCyte 12 and 12HT Systems



How it Works The guava easyCyte systems use patented, microcapillary, laser-based technology capable of detecting mammalian and microbial cells and beads. A sample of fluorescently labeled cells is aspirated into a uniquely proportioned microcapillary flow cell. A red or blue diode laser excites the cells and each cell emits signals that are individually detected by photomultipliers and a photo diode. Guava software modules show all relevant data and results immediately.

Like all guava systems, the easyCyte HT family of flow cytometry systems uses patented microcapillary technology that eliminates the need for sheath fluid and enables absolute cell counts without the need for reference beads. Complemented by our intuitive software, the systems provide flexible data collection and analysis with the option to use optimized modules or design your own assays.

■ Anatomy of the guava easyCyte™ Systems

▸ guava easyCyte – High Throughput Sampling

The guava easyCyte flow cytometry systems are uncomplicated instruments that deliver the power of multiplexed cell analysis—right on your benchtop. The culmination of over a decade of flow cytometry expertise, these instruments use minimal sample, generate less waste, and are easier to use and maintain than traditional flow cytometers—all while providing the power you need in the most compact format available. These advantages are made possible by our patented microcapillary flow cell technology.

▸ guava easyCyte – Single Sampling

guava easyCyte™ System Features

System	easyCyte™ 5	easyCyte™ 5HT	easyCyte™ 6-2L	easyCyte™ 6HT-2L	easyCyte™ 8	easyCyte™ 8HT	easyCyte™ 12	easyCyte™ 12HT
Catalogue No.	**0500-5005**	**0500-4005**	**0500-5007**	**0500-4007**	**0500-5008**	**0500-4008**	**0500-5012**	**0500-4012**
Violet (405 nm) Laser							✓	✓
Blue (488 nm) Laser	✓	✓	✓	✓	✓	✓	✓	✓
Red (642 nm) Laser			✓	✓	✓	✓	✓	✓
FSC	✓	✓	✓	✓	✓	✓	✓	✓
SSC	✓	✓	✓	✓	✓	✓	✓	✓
Blue-V (448/50 nm)							✓	✓
Green-V (525/30 nm)							✓	✓
Yellow-V (583/26 nm)							✓	✓
Red-V (695/50 nm)							✓	✓
Green-B (525/30 nm)	✓	✓	✓	✓	✓	✓	✓	✓
Yellow-B (583/26 nm)	✓	✓	✓	✓	✓	✓	✓	✓
Red-B (695/50 nm)	✓	✓	✓	✓	✓	✓	✓	✓
NIR-B (785/70 nm)					✓	✓	✓	✓
Red-R (661/15 nm)			✓	✓	✓	✓	✓	✓
NIR-R (785/70 nm)					✓	✓	✓	✓
Microcapillary Fluidics	✓	✓	✓	✓	✓	✓	✓	✓
Direct, Absolute Cell Counts	✓	✓	✓	✓	✓	✓	✓	✓
Automation-plate and tubes		✓		✓		✓		✓
Mixing		✓		✓		✓		✓
Dell® Laptop	✓	✓	✓	✓	✓	✓	✓	✓
InCyte™ Software	✓	✓	✓	✓	✓	✓	✓	✓
Digital Signal Processing	✓	✓	✓	✓	✓	✓	✓	✓

Spectral Bands and Applicable Dyes

Blue (448/50 nm)	Green (525/30 nm)	Yellow (583/26 nm)	Red (661/15 nm)	Red (695/50 nm)	NIR (785/70 nm)
DAPI	Alexa Fluor® 430	Pacific Orange™ dye	Alexa Fluor® 647	eFluor® 650	PE-Alexa Fluor® 750
Hoescht 33258	Pacific Green	Brilliant Violet™ dye	APC	Brilliant Violet™ dye	Propidium Iodide
Alexa Fluor® 405	Brilliant Violet™ dye	Qdot® 565	CD647	Qdot® 705	PE-Cy-7
Marnia Blue® dye	Qdot® 525	Qdot® 585	Cy5	7-AAD	APC-Cy7
Pacific Blue™ dye	Qdot® 545	Alexa® 555	Qdot® 655	Propidium Iodide	APC-Alexa Fluor® 750
Cascade Blue® dye	FITC	Alexa® 568	DRAQ5	PE-Alexa Fluor® 647	
LIVE/DEAD® Violet	GFP	CF555	Ethidium Bromide	PE-Alexa Fluor® 700	
DyLight® 405	Alexa Fluor® 488	PE-B, PE-R	Ethidium Homodimer	PE-Cy5	
eFluor® 450	CF488	Qdot® 565	SYTOX® Red	PE-Cy5.5	
Zombie Aqua™ dye	FAM	Acridine Orange	TO-PRO®-3	PE-Texas Red® dye	
Brilliant Violet™ dye	Qdot® 525	dsRED	TOTO-3	PerCP	
	Acridine Orange	Ethidium Bromide	DilC1(5)	PerCP-Cy5.5	
	SYBR® Green	SYBR® Green	MitoSense Red	Qdot® 705	
	SYTOX Green®	SYTOX® Orange	BODIPY 650/665	DRAQ5	
	JC-1	JC-1		Ethidium Bromide	
	BODIPY-FL	TMRE		Ethidium Homodimer	
	Calcein	TRMR		LDS-751	
	CFSE	CFSE		Nile Red	
	Oregon Green® dye	Nile Red			

For a complete list please see: www.merckmillipore.com/guava

405 nm Laser	488 nm Laser	642 nm Laser

Instrument Service Plan Options

Service Plan Features*	One-Year Warranty	Service Total Plan	Preventative Maintenance Plan
Travel, Labor & Expenses to Perform Service	✓	✓	
Instrument Installation at Customer Site	✓	✓	
Coverage of Parts	✓	✓	
Preventative Maintenance		✓	✓
Factory Trained & Authorized Service Engineers	✓	✓	✓
Technical Support Services	✓	✓	✓
Priority Scheduling		✓	

* No service contract is required for a one-time service request.

Software

Your specific research needs are always changing and Merck Millipore's guava software is uniquely adaptable to accommodate you at every level. The guavaSoft application-specific modules have plug-and-play formats designed for our optimized reagents. For more flexible, user-defined formats, InCyte software delivers many high-level features which uniquely enable easy visualization of data in a broad biological context. All our software modules use the same intuitive user interface, to make it easy to switch from one format to another. You can export data quickly to spreadsheets or any thirdparty analysis format. Moreover, our software packages can enable 21 CFR part 11 compliance.

■ InCyte: Intuitive

InCyte software brings a new level of analytical power to flow cytometry. It is the first solution designed to empower every user to draw conclusions about the biological significance of data. Its intuitive, easy-to-use interface makes it possible to visualize and compare up to eight data sets at once, with drag and drop features to simplify the set up of gating strategies. Many high-level analysis features are already built in. Entire experiments can now be analyzed and viewed at once, in less time than it usually takes to analyze a single sample. One of its uniquely powerful benefits is the ability to display comparative results and the experiment level, using features like heat maps and IC50/ EC50 curves which allow easy target identification. Automated compensation reduces the amount of time to analyze complex multicolor assays by providing an automatic correction of the spectral overlap of simultaneously analyzed dyes. As a result, InCyte software can function as the primary data acquisition and analysis package for the instrument.

Drag-and-drop gating allows selection of specific populations for further analysis, with the ability to highlight groups using color back-gating

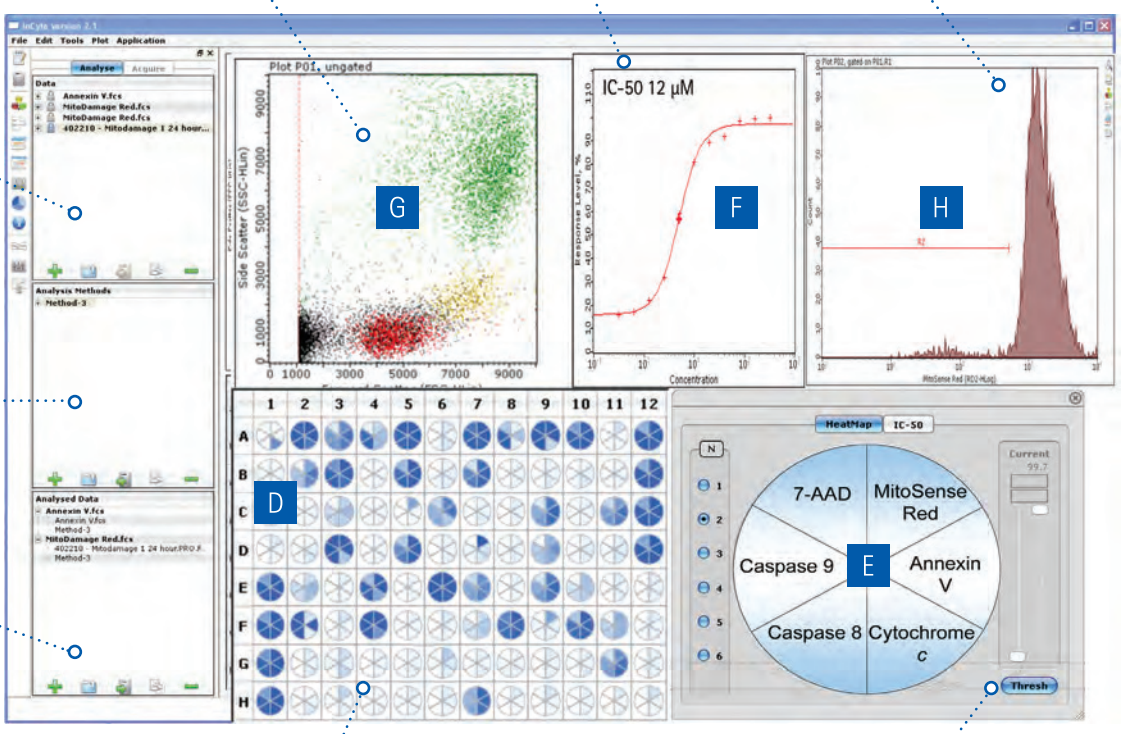
IC_{50} curves show inhibition range of a dose-response curve

View up to 11 plots all at once, with real-time plot adjustments

Organize acquired data in this panel

Easily create analysis templates

Quickly link to and review previously analyzed data



Heat mapping allows rapid visualization of up to 6 parameters at once, within a single plate or across multiple experiments

Construct heat maps or EC_{50}/ IC_{50} curves by selecting groups of data and using slider bars to set cut-offs or threshold values

Applications

Weather you're studying cancer, stem cells, immune function, cell signaling, or something else of cell biology, you'll quickly be an expert using Merck Millipore's assay kits that are designed and optimized for use in flow cytometry. We've taken the guess work out of assay development so you can focus on your results. All you need are cells and a research question; our assay kits will do the rest, and you'll have data before your cells are ready to split again.

Cell Health
- Cell Counting & Viability
- Cell Cycle
- DNA Damage
- Mitochondrial Analysis
- Apoptosis

Cell Signaling
- MAPK Pathway
- EGFR Pathway
- PI3/Akt/mTOR Pathway
- Jak/STAT Pathway
- Chemokine

Stem Cells
- Embryonic Stem Cell (Human/Mouse)
- Neural Stem Cell (Rodent)

Immunology
- Regulation T-Cell
- Phenotype Markers

Others
- SpaceCraft
- Radiation Exposure
- Biofuels (Microalgae)
- Coal to Natural Gas
- Fermentation

Milli-Mark® Conjugated Antibodies

■ Cell Health - Cell Counting & Viability

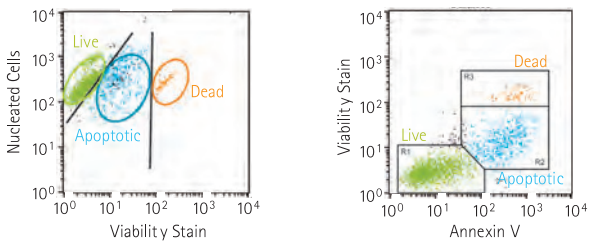


Figure 1.

The blue population of cells show a significant amount of annexin V staining (right plot), indicating that intermediate levels of staining with the viability dye correlates with apoptosis.

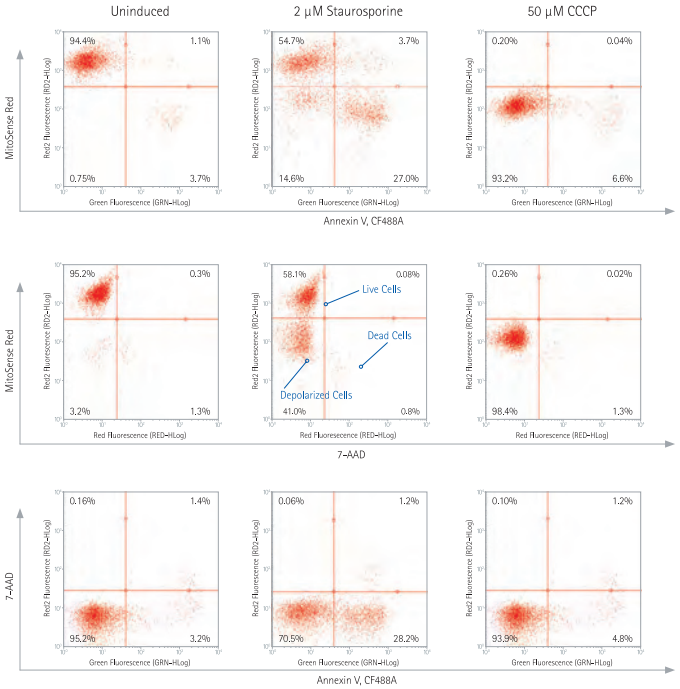
guava ViaCount® Assay Kit

Advantages

Assays:
- Simple no-wash, mix-and-read procedure
- Counts up to 10 times faster than manual methods
- More reproducible than traditional tests

Samples:
- Uses small samples in tubes or a 96-well plate
- Handles low-density and small-volume cell samples
- Works with adherent or suspended cells and mammalian and insect cells

■ Cell Health - Apoptosis



FlowCellect MitoDamge Kit

Advantages

Assay:
- Simple no-wash, mix-and-read format
- Provides direct, absolute cell counts as well as population percentages
- Ease of use with incite software
- Suitable for use with other flow cytometers

Samples:
- Uses small samples in tubes or a 96-well plate
- Handles low-density and small-volume cell samples
- Works with adherent or suspended cells and mammalian and insect cells

Figure 2.

Dot plots depicting Jurkat cells stained using the MitoDamage kit. Jurkat cells uninduced, induced to apoptosis with 2 µM staurosporine or with 50 µM CCCP, then stained using the MitoDamage kit. Plots show the percentage of positive cells for:

1st row: Apoptosis (Annexin V binding) and mitochondrial membrane potential change

2nd row: Cell death and mitochondrial membrane potential change

3rd row: Apoptosis and cell death. Data reports that 2 µM staurosporine induces apoptosis in Jurkat cells, and that 50 µM CCCP depolarizes the mitochondrial membrane, but neither condition is sufficient for cell membrane permeabilization and death.

■ Cell Signaling

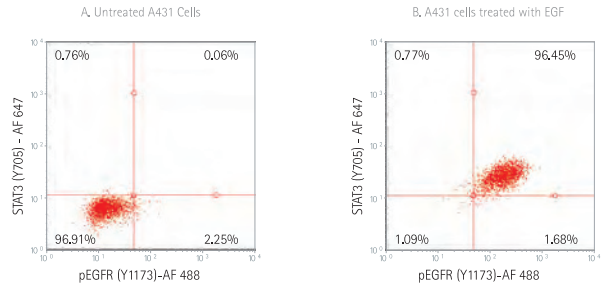


Figure 3.

Analyzed Dual Parameter Data: Dot plots depicting Untreated A431 cells stained for both pEGFR-Alexa Fluor® 488 and pSTAT3-Alexa Fluor 647 (Plot A) and A431 cells treated with 100 ng/mL of EGF and then stained simultaneously with both pEGFR-Alexa Fluor 488 and pSTAT3-Alexa Fluor 647. 96.45% of the treated cells are double positive indicating activation of STAT3 through EGFR (Plot B).

FlowCellect EGFR/STAT3 Pathway Activation Detection Kit

Advantages

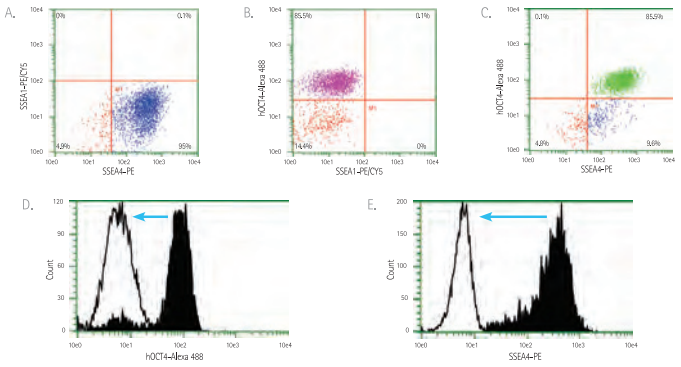
Assays:

- Directly conjugated antibodies optimized for multi-color flow cytometry application
- Kit includes optimized fixation, permeabilization, wash and flow buffers
- Direct, absolute counts and percentages
- Suitable for use with other flow cytometers

Samples:

- Contains everything you need to run 25 samples
- Works with adherent and suspended cells

■ Stem Cells



Human embryonic stem cells defined with the FlowCellect Human Embryonic Stem Cell Nuclear Marker Characterization Kit (FCHEC25102).

Figure 4.

Oct-4 and SSEA-4 are both expressed on undifferentiated human embryonic stem cells. SSEA-1 is not expressed on human stem cells, but can be expressed upon treatment with retinoic acid. In this test, cells are labeled as expected: negative for SSEA-1 (A and B), positive for SSEA-4 (A and C), and positive for Oct-4 (B and C). Histogram overlays show that human stem cell markers Oct-4 (D) and SSEA-4 (E) are both expressed in H1 human embryonic stem cells (shaded histogram) but are not expressed in differentiated ENStem-A™ neural progenitor cells (unshaded histogram).

FlowCellect Human Embryonic Stem Cell Characterization Kit

Advantages

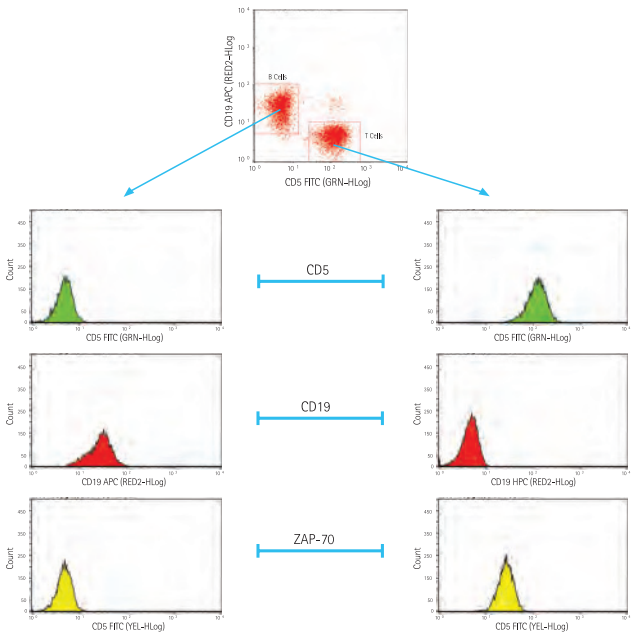
Assay:

- Optimized antibodies and isotype controls are directly conjugated and validated for flow cytometry
- Contains everything you need to run 25 samples
- Less than two hours of hands-on time

Samples:

- Embryonic or neural stem cells
- Human, murine or rat samples
- Included cell strainer prevents cell clumping

■ Immunology



FlowCellect Human Lymphocyte ZAP-70 Characterization Kit

Advantages

Assay:

- Removes multiplexing optimizations
- Highly reproducible
- Minimized assay development time
- Enable novice users to perform complex analysis
- Suitable for use with other flow cytometers

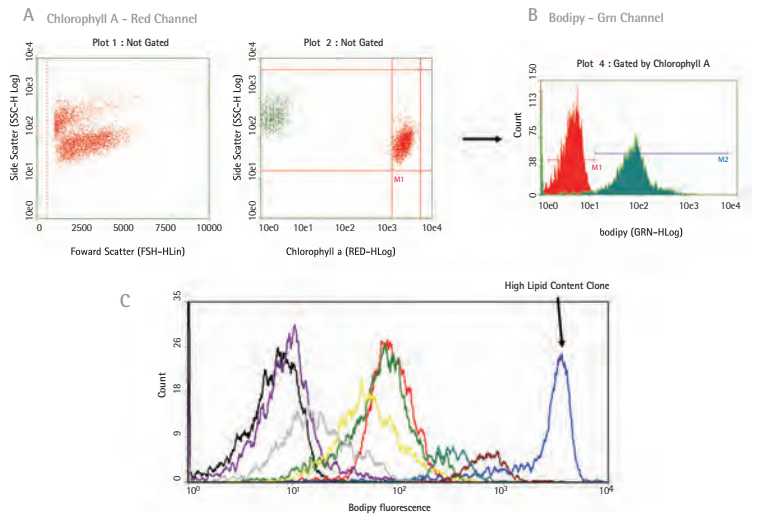
Samples:

- Designed to run 25 tests

Figure 5.

Mixture of Roamos (B cell line) and Jurkat (T cell Line) cells were used as a model system. The cells were then stained with CD5-FITC, CD19-APC, and ZAP-70 PE. A dot plot showing CD5 vs. CD19 is used to determine the B cells from T cells and is used to draw two gates which allow for the analysis of the expression levels of all three markers within each population.

Biofuels (Microalgae)



Lipid Measurement in Algae

Selection of high lipid-producing algal strains using low cost production methods are prerequisites for a sustain - able and economically-viable algal biomass fuel industry. Algal monitoring requires the rapid and timely assessment

- Cell counts
- Chlorophyll & Lipid content
- Traditional methods for algal strain selection

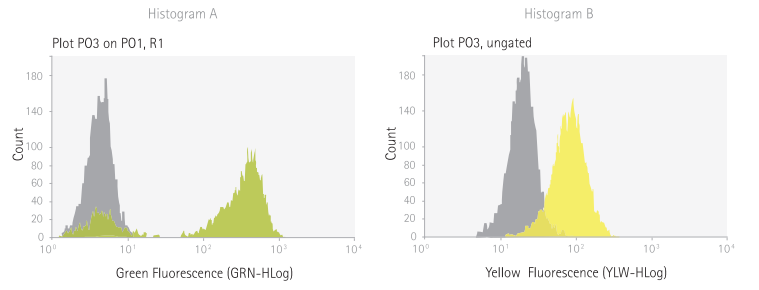
Guava's bench-top cytometric platforms allow for simple, rapid analysis and selection of algal strains utilizing scat - ter and fluorescence properties.

Figure 6.
Bodipy Measurement of Lipid Content in Chlorophyll A Positive Algae

A. Characterization by Morphology and Chlorophll A Properties
B. Gate on Chlorophyll A positive cells Quantify Bodipy content using Histogram
C. Histograms of BODIPY green fluorescence intensity of a variety of algal strains showing numbers of cells with variations in intracellular neutral lipid contents.

FlowCellect® kits and Milli-Mark® antibodies

Merck Millipore's FlowCellect® kits and Milli-Mark® conjugated primary antibodies are fully optimized for fast, easy, and accurate multipara-metric flow cytometry. We've taken the guesswork out of assay development so you can focus on your results. Assay components are highly stable so you can run samples sequentially or as a large group, without compromising results. Our expanding portfolio of kits is validated for use on guava easyCyte™ flow cytometers, including our NEW guava easyCyte™ 12 system! All kits are also cross-platform tested on traditional sheath-based flow cytometers. All reagents (except cells) are included.
The combination of user friendly software with optimized, turnkey assay kits provides many benefits, including reduced sample preparation time, shortened assay development, low-cost multiplexing capability without the need for compensation, and ease of detection. Or you can build your own assay with any compatible flow cytometry reagents, and use automated compensation following acquisition as the primary data acquisition and analysis package for the instrument.



Advance your flow cytometry analysis with Merck Millipore's growing selection of directly conjugated primary antibodies. As a part of our complete benchtop flow cytometry solution including instruments, software, service, and reagents, Milli-Mark fluorescently-labeled antibodies are specifically designed, optimized and vali - dated for flow cytometry applications

Figure 7.
Histogram A: Flow cytometry analysis using anti-phospho-Histone H3 (Ser10). Jurkat cells were treated with 50 nM Calyculin A for 4 hours at 37 °C (green), or untreated (grey). Histogram B: HeLa cells were either treated with etoposide (yellow) or untreated (grey) and then stained with anti-phospho ATM(1981)-PE.

Ordering Information

Description	Cat. No.
guava easyCyte™ Systems	
Single Sampling Instruments	
guava easyCyte™ 5 Base System	0500-5005
guava easyCyte™ 6-2L Base System	0500-5007
guava easyCyte™ 8 Base System	0500-5008
guava easyCyte™ 12 Base System	0500-5012
High Throughput Sampling Instruments	
guava easyCyte™ 5HT Base System	0500-4005
guava easyCyte™ 6HT-2L Base System	0500-4007
guava easyCyte™ 8HT Base System	0500-4008
guava easyCyte™ 12HT Base System	0500-4012
Software Modules for guava easyCyte™ Systems	
guavaSoft™ Software Package (includes InCyteTM, Express Pro, Express Plus and guavaSuiteTM modules)	0500-4115
InCyte™ Software Module	0500-4120
guava® Express Pro Software Module	0500-4125
guavaSuite™ Software Modules	0500-4130

Description	Cat. No.
Service plans for guava easyCyte™ HT Systems	
Service Total (ST) Plans	
guava easyCyte™ 5HT ST 1 year plan, at time of purchase	0500-4300
guava easyCyte™ 5HT ST 1 year plan, after purchase	0500-4305
guava easyCyte™ 5HT ST 2 year plan	0500-4310
guava easyCyte™ 6HT/2L ST 1 year plan, at time of purchase	0500-4330
guava easyCyte™ 6HT/2L ST 1 year plan, after purchase	0500-4335
guava easyCyte™ 6HT/2L ST 2 year plan	0500-4340
guava easyCyte™ 8HT ST 1 year plan, at time of purchase	0500-2870
guava easyCyte™ 8HT ST 1 year plan, after purchase	0500-2875
guava easyCyte™ 8HT ST 2 year plan	0500-2880
guava easyCyte™ 12HT ST 1 year plan, at time of purchase	0500-3870
guava easyCyte™ 12HT ST 1 year plan, after purchase	0500-3875
guava easyCyte™ 12HT ST 2 year plan	0500-3880
Preventative Maintenance (PM) Plans	
guava easyCyte™ 5HT PM 1 year plan, at time of purchase	0500-4285
guava easyCyte™ 5HT PM 1 year plan, after purchase	0500-4290
guava easyCyte™ 5HT PM 2 year plan	0500-4295
guava easyCyte™ 6HT/2L PM 1 year plan, at time of purchase	0500-4315

Description	Cat. No.
guava easyCyte™ 6HT/2L PM 1 year plan, after purchase	0500-4320
guava easyCyte™ 6HT/2L PM 2 year plan	0500-4325
guava easyCyte™ 8HT PM 1 year plan, at time of purchase	0500-4270
guava easyCyte™ 8HT PM 1 year plan, after purchase	0500-4275
guava easyCyte™ 8HT PM 2 year plan	0500-4280
guava easyCyte™ 12HT PM 1 year plan, at time of purchase	0500-6270
guava easyCyte™ 12HT PM 1 year plan, after purchase	0500-6275
guava easyCyte™ 12HT PM 2 year plan	0500-6280
Service plans for guava easyCyte™ Single Sample Loader Systems	
Service Total (ST) Plans	
guava easyCyte™ 5 ST 1 year plan, at time of purchase	0500-5300
guava easyCyte™ 5 ST 1 year plan, after purchase	0500-5305
guava easyCyte™ 5 ST 2 year plan	0500-5310
guava easyCyte™ 6-2L ST 1 year plan, at time of purchase	0500-5330
guava easyCyte™ 6-2L ST 1 year plan, after purchase	0500-5335
guava easyCyte™ 6-2L ST 2 year plan	0500-5340
guava easyCyte™ 8 ST 1 year plan, at time of purchase	0500-5870
guava easyCyte™ 8 ST 1 year plan, after purchase	0500-5875
guava easyCyte™ 8 ST 2 year plan	0500-5880
guava easyCyte™ 12 ST 1 year plan, at time of purchase	0500-6870
guava easyCyte™ 12 ST 1 year plan, after purchase	0500-6875
guava easyCyte™ 12 ST 2 year plan	0500-6880
Preventative Maintenance (PM) Plans	
guava easyCyte™ 5 PM 1 year plan, at time of purchase	0500-5285
guava easyCyte™ 5 PM 1 year plan, after purchase	0500-5290
guava easyCyte™ 5 PM 2 year plan	0500-5295
guava easyCyte™ 6-2L PM 1 year plan, at time of purchase	0500-5315
guava easyCyte™ 6-2L PM 1 year plan, after purchase	0500-5320
guava easyCyte™ 6-2L PM 2 year plan	0500-5325
guava easyCyte™ 8 PM 1 year plan, at time of purchase	0500-5270
guava easyCyte™ 8 PM 1 year plan, after purchase	0500-5275
guava easyCyte™ 8 PM 2 year plan	0500-5280
guava easyCyte™ 12 PM 1 year plan, at time of purchase	0500-6370
guava easyCyte™ 12 PM 1 year plan, after purchase	0500-6375
guava easyCyte™ 12 PM 2 year plan	0500-6380
Installation Qualification and Operation Qualification (IQ/OQ) Services	
IQ/OQ guava easyCyte™ Single Loader System, customer performed	8000-1995
IQ/OQ guava easyCyte™ Single Loader System, Merck Millipore performed	8000-1996
IQ/OQ guava easyCyte™ HT System, customer performed	8000-1997
IQ/OQ guava easyCyte™ HT System, Merck Millipore performed	8000-1998
Additional parts for guava easyCyteTM Systems	
Guava Instrument Cleaning Fluid (ICF); 100 mL	4200-0140
FlowCell for guava easyCyte™ HT Systems*	0500-2260
FlowCell for guava easyCyte™ Single Sample Loader Systems*	0500-2270
Waste Bottle for guava easyCyte™ Systems	0110-3020
Cleaning Bottle Assay for guava easyCyte™ Systems	0110-3030
Flow Cell Removal Tool	6000-2410
Waste Bottle Assay for guava easyCyte™ HT Systems	0110-5790
Cleaning Bottle for guava easyCyte™ HT Systems	0110-5780
Tubing for waste/cleaning bottles	2500-0060
FlowCellect® Kits	
ViaCount® Assay Kits	
Guava ViaCount® Reagent (100 Tests)	4000-0040
Guava ViaCount® Reagent (600 Tests)	4000-0041
Guava ViaCount® Flex Reagent Kit (100 Tests)	4500-0110
Guava ViaCount® Flex Reagent Kit for Challenging Samples***	4700-0060
Guava ViaCount® Cell Dispersal Reagent (100 Tests)	4700-0050
Cell Cycle Kits	
FlowCellect® Bivariate Cell Cycle Kit for DNA Replication Analysis (25 Tests)	FCCH025102
FlowCellect® Bivariate Cell Cycle Kit for G2/M Analysis (25 Tests)	FCCH025103
Guava® Cell Cycle Reagent (100 Tests)	4500-0220
DNA Damage Kits	
FlowCellect® Multi-Color DNA Damage Response Kit*	FCCH025104
FlowCellect® DNA Damage Histone H2A.X Dual Detection Kit*	FCCS025153
FlowCellect® Cell Cycle Checkpoint H2A.X DNA Damage Kit*	FCCH025142
FlowCellect® Cell Cycle Checkpoint ATM DNA Damage Kit*	FCCH025143
FlowCellect® Histone H2A.X Phosphorylation Assay Kit**	FCCS100182

*Qty=25 Tests; ** Qty=100 Tests; *** Qty=500 Tests

Description	Cat. No.
Mitochondrial Health Kits	
FlowCellect® MitoPotential Red Kit (100 Tests)	FCCH100105
FlowCellect® MitoDamage Kit (100 Tests)	FCCH100106
FlowCellect® MitoLive Kit (100 Tests)	FCCH100107
FlowCellect® MitoStress Kit (100 Tests)	FCCH100109
FlowCellect® Cytochrome c Kit (100 Tests)	FCCH100110
FlowCellect® Oxidative Stress Characterization Kit (25 Tests)	FCCH025111
FlowCellect® MitoCaspase 3/7 Kit (100 Tests)	FCCH100178
Guava® Mitochondrial Depolarization Assay Kit (100 Tests)	4500-0250
Apoptosis Kits	
Early Apoptosis Kits	
FlowCellect® Annexin Red Kit (100 Tests)	FCCH100108
Guava Nexin® Reagent (100 Tests)	4500-0450
Guava Nexin® Reagent (500 Tests)	4500-0455
Mid Apoptosis Kits	
Guava® MultiCaspase SR Kit (100 Tests)	4500-0500
Guava® Caspase 9 SR Kit (100 Tests)	4500-0520
Guava® MultiCaspase FAM Kit (100 Tests)	4500-0530
Guava® Caspase 3/7 FAM Kit (100 Tests)	4500-0540
Guava® Caspase 8 FAM Kit (100 Tests)	4500-0550
Guava® Caspase 9 FAM Kit (100 Tests)	4500-0560
Guava® MultiCaspase SR and Caspase 3/7 FAM Kit (100 Tests)	4500-0570
Guava® MultiCaspase SR and Caspase 8 FAM Kit (100 Tests)	4500-0580
Guava® MultiCaspase SR and Caspase 9 FAM Kit (100 Tests)	4500-0590
Guava® Caspase 9 SR and Caspase 3/7 FAM Kit (100 Tests)	4500-0630
Guava® Caspase 9 SR and Caspase 8 FAM Kit (100 Tests)	4500-0640
Guava® Caspase 9 SR and MultiCaspase FAM Kit (100 Tests)	4500-0650
Late Apoptosis Kit	
Guava® TUNEL Kit (100 Tests)	4500-0121
Apoptosis Signaling Kit	
FlowCellect® Bcl-2 Activation Dual Detection Kit (25 Tests)	FCCS025108
Autophagy	
FlowCellect® GFP-LC3 Reporter Autophagy Assay Kit (CHO)**	FCCH100170
FlowCellect® GFP-LC3 Reporter Autophagy Assay Kit (U2OS)**	FCCH100181
FlowCellect® RFP-LC3 Reporter Autophagy Assay Kit**	FCCH100183
FlowCellect® Autophagy LC3 Antibody-based Assay Kit**	FCCH100171
Cell Signaling	
FlowCellect Kits: MAPK Pathway	
FlowCellect PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection Kit (25 Tests)	FCCS025100
FlowCellect EGFR/MAPK Pathway Activation Detection Kit (25 Tests)	FCCS025101
FlowCellect MAPK Activation Dual Detection Kit (25 Tests)	FCCS025106
FlowCellect p38 Stress Pathway Activation Detection Kit (25 Tests)	FCCS025132
FlowCellect Kits: EGFR Pathway	
FlowCellect EGFR/MAPK Pathway Activation Detection Kit (25 Tests)	FCCS025101
FlowCellect EGFR RTK Activation Dual Detection Kit (25 Tests)	FCCS025107
FlowCellect EGFR/STAT3 Pathway Activation Detection Kit (25 Tests)	FCCS025111
PI3 / Akt / m-TOR Pathway	
FlowCellect PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection Kit (25 Tests)	FCCS025100
FlowCellect PI3K Activation Dual Detection Kit (25 Tests)	FCCS025105
FlowCellect PI3K-mTOR Signaling Cascade Mapping Kit (25 Tests)	FCCS025210
Jak / STAT Pathway	
FlowCellect EGFR/STAT3 Pathway Activation Detection Kit (25 Tests)	FCCS025111
FlowCellect Multi-STAT Activation Profiling Kit (25 Tests)	FCCS025550
FlowCellect STAT1 Activation Dual Detection Kit (25 Tests)	FCCS025142
FlowCellect STAT3 Activation Dual Detection Kit (25 Tests)	FCCS025143
Multiple Pathway	
FlowCellect Src Activation Dual Detection Kit (25 Tests)	FCCS025154
FlowCellect PLC- γ1 Activation Dual Detection Kit (25 Tests)	FCCS025145
Apoptosis Signaling Pathway	
FlowCellect Bcl-2 Activation Dual Detection Kit (25 Tests)	FCCS025108
Chemokine Receptor Kits	
FlowCellect Chemokine Receptor CCR2B Surface Expression Quantification Kit (25 Tests)	FCCR200411
FlowCellect Chemokine Receptor CCR4 Surface Expression Quantification Kit (100 Tests)	FCCR400413
Chemokine Receptor CCR6 Surface Expression Quantification Kit (100 Tests)	FCCR600414
FlowCellect Chemokine Receptor CCR7 Surface Expression Quantification Kit (100 Tests)	FCCR700415

Cell Analyzer

Muse™ Cell Analyzer

Be inspired by innovation at your side. The Muse™ Cell Analyzer delivers accurate assessments of cell viability, apoptosis cell cycle and much more in just minutes, revolutionizing the way you analyze cells. With the Muse™ Cell Analyzer's microcapillary and miniaturized optics, sleek, touchscreen operation and simple sample prep, you can make faster, more accurate decisions about your experiments, for more productive research.

The Muse® Cell Analyzer is a highly intuitive, compact instrument for the fluorescence-based, 3-parameter analysis. It is designed to make flow cytometry easy, convenient and accessible for all researchers. The user interface is specifically tailored for streamlined applications, so that you can move from sample setup to analysis and results in just a few minutes. Convenient Muse® "Mix-and-Read" Assays enable samples to be prepared in a simple step and then loaded onto the Muse® instrument for fast, easy analysis. The Muse® instrument has an integrated computer and software for data acquisition and analysis of optimized Muse® Assays. It is versatile enough to analyze both suspension and adherent cells 2-60 microns in diameter.

- Highly quantitative data at the single cell level
- Simple, effortless operation
- Intuitive software and touchscreen user interface
- Rapid setup and analysis
- Optimized Muse® assays
- Compact (20 cm x 25 cm) and affordable

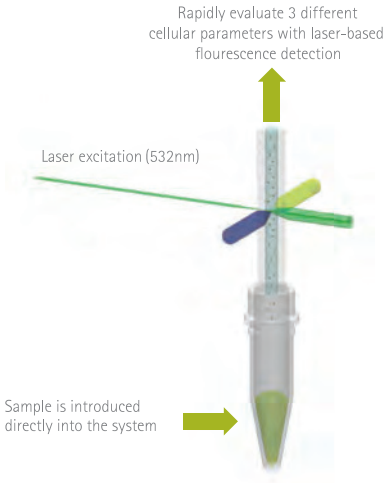
Technology

Measure more accurately and reliably

The Muse™ Cell Analyzer uses patent-pending, miniaturized fluorescent detection and microcapillary technology to deliver truly accurate, precise and quantitative cell analysis compared to other methods. Versatile enough to analyze both suspension and adherent cells 2-60 µm in diameter, the Muse™ Cell Analyzer provides greater accuracy and precision than other analysis methods.

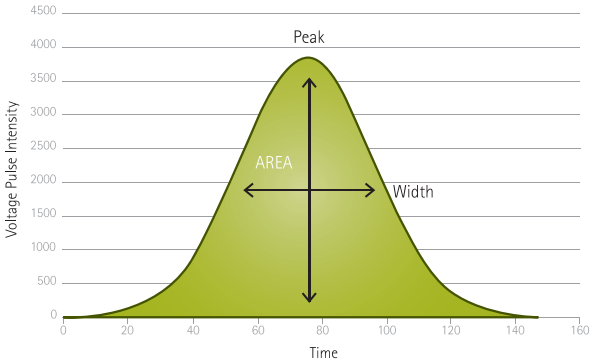
■ Laser-based Fluorescence Detection

The system delivers high-performance cell analysis using a microcapillary and miniaturized optics which occupy one-tenth the space of a typical cytometer. Laser-based fluorescence detection of each cell event can evaluate up to 3 cellular parameters – cell size (forward scatter) and 2 colors (detected in the red and/or yellow channels). A green diode laser is used for excitation, and a uniquely designed series of retro-reflective lenses provide maximum light capture and sensitivity. As a result, Muse™ provides more quantitative results than imagingbased systems, which only examine up to 2 parameters, are time consuming and ultimately provide less quantitative data.



■ A Basis for Sensitive Detection

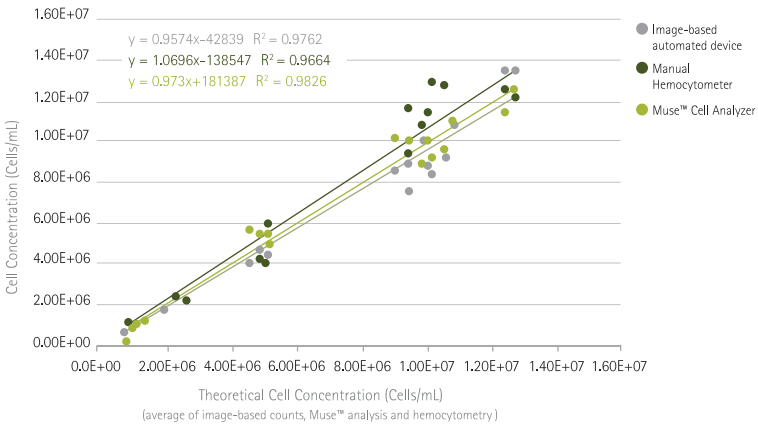
Fluorescence emitted by a cellular event is detected as a voltage pulse, described in diagram above. The width and height are proportional to the amount of fluorescence intensity for each cell event, and this leads to highly quantitative measurements of labeled cells.



Performances

■ Analysis is Accurate

The Muse™ Cell Analyzer counts cells more accurately than manual hemocytometry or image-based automated analysis. Multiple adherent and suspension cell types (MCF-7, K562, Hb, CHO-K1, and Jurkat cells) were counted using the methods shown. Cell counts from all three methods were averaged to obtain "theoretical cell concentration". Each point represents average of 3 replicates, and each data series was fit with linear regression. Muse™ cell analysis data were correlated with theoretical concentration with slope closest to 1, indicating superior accuracy.



Analysis is Precise

The Muse™ Cell Analyzer counts cells and measures viability more precisely with smaller coefficients of variation (%CV) than manual hemocytometry or image-based automated analysis. While image-based automated counting methods and manual hemocytometry displayed broader ranges of %CVs, the Muse™ Cell Analyzer exhibited a narrow range of %CVs and consistently provided %CVs less than 10% over the entire range of samples tested. Higher %CVs were observed for the Trypan blue-based methods, particularly at lower cell concentrations.

Analysis Method	Cell Concentration		Viability	
	Average %CV	%CV Range	Average %CV	%CV Range
Muse™ Cell Analyzer	4.0%	0.3–8.8%	2.2%	0.4–5.6%
Image-based Automated Counter	9.2%	1.2–23.3%	3.7%	0.8–12.1%
Manual Hemocytometer	6.3%	0.5–15.3%	4.5%	0.5–9.2%

Table 1.
The Muse™ Cell Analyzer provides superior precision for cell concentration and viability measurements, compared to Trypan blue-based analyses. Data are based on triplicate measurements of 30 cellular samples from suspension and adherent cell lines at multiple concentrations and viabilities.

Analysis is Reliable

The Muse™ Cell Analyzer performs with high linearity across multiple cell lines and a wide sample concentration range. The concentration results for serial dilutions of 5 representative cell lines are shown, which include both adherent and suspension cells. Each point represents the average of three samplings. Expected cell concentrations were calculated by measuring the stock sample concentration with the Muse™ Cell Analyzer, then dividing by the dilution factor to obtain theoretical concentrations of diluted samples. Data show the comparison of observed vs. expected cell concentrations.

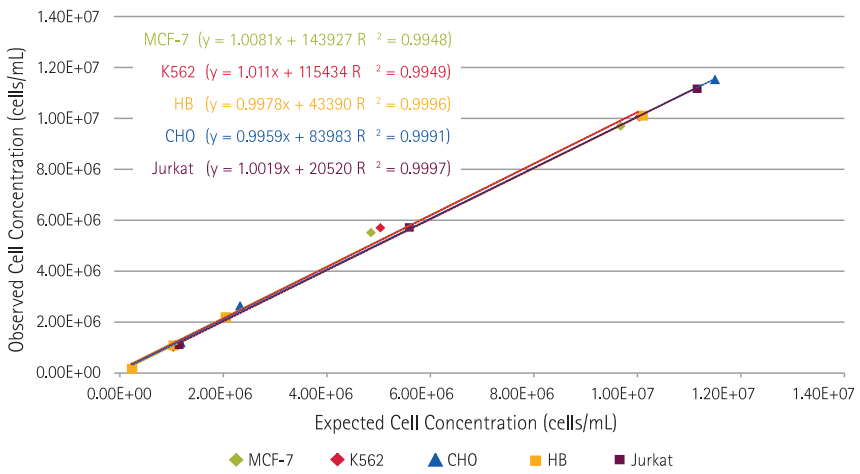


Figure 1.
The data show the comparison of observed vs. expected cell concentration results for serial dilutions of 5 representative cell lines shown, which include both adherent and suspension cells. Each point represents the average of three samplings.

■ Comparison of Muse counting compared to other counting systems

We compared the accuracy of the Muse™ Count & Viability Assay with other methods that provide count and viability information:

- Traditional methods of cell counting that utilize Trypan blue staining such as manual hemocytometer counts
- Automated image-based analysis of Trypan blue stained samples.

	Muse™ Cell Analyzer	Manual Hemocytometer	Automated Imaging-based Counting Device
Sample format required for acquisition	Tube-based	Slide-based	Slide-based
Staining type	Fluorescent dyes	Trypan blue	Trypan blue
Degree of operator bias	Minimal	Significant bias	None
Variability in number of cells counted	No variability	Number of cells counted is concentration-dependent and may vary between samples	Number of cells counted is not clear, concentration-dependent
Number of cells counted	More cells, increased statistical significance	Fewer cells	Fewer cells
Acquisition speed	1–2 minutes	Slower due to manual counting	~ 1 minute
Flexibility in sample reading/ analysis	Greater flexibility in sample read time after staining	Samples must be analyzed soon after staining	Samples must be analyzed soon after staining
Data export features	Advanced export features, reanalysis of data, allows for documentation of report; Excel® file export option	Lost after read; manually writtendown results	Exportable to .csv file – only counts exported

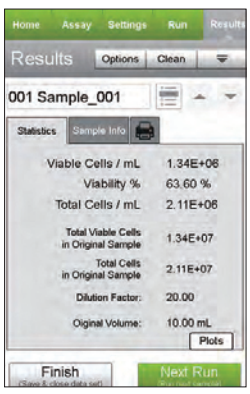
Table 2. Comparison of Muse counting compared to other counting systemsities.

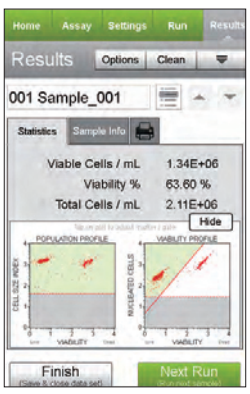
Applications

The Muse™ Cell Analyzer makes 3-parameter, quantitative cell analysis unbelievably simple. For the assays you rely on most, we've developed optimized kits, validated for robust performance on the Muse™ Cell Analyzer. Typical cell preparation protocols have been condensed and simplified, so sample preparation is fast and easy. You don't need to optimize any software settings – the Muse® instrument calculates all gating parameters and thresholds for you. Just prepare your sample with Muse™ all-in-one reagents, load on the instrument, and follow the easy, guided menus on the touchscreen to get your results. Results are displayed in both graphical and statistical formats specific to each application, making analysis unambiguous. Spend less time with experimental setup, avoid reagent waste and save money – we've done all the work for you. Easy raw data and Excel® export features allow for archiving of results and additional analysis. Export into third party software programs such as FlowJo or Modfit is possible using the FCS converter. We are continually releasing new Muse® assay modules and kits. Please visit our web page for the most up-to-date listing of Muse® Assays: **www.merckmilllipore.com/muse**

■ Muse® Assays for Cell Health Analysis

▶ Count & Viability Assay

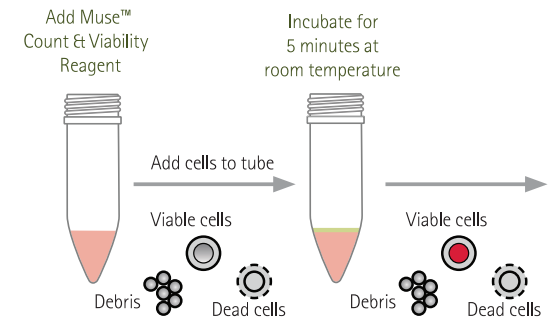




Muse™ Cell Count and Viability Kit

Absolute total cell counts and viability of dead and dying cells based on differential permeability of two DNA-binding dyes.

One of the dyes is membrane-permeant and stains all cells with a nucleus. The second dye only stains dying or dead cells, whose membranes have been compromised. This combination allows for the discrimination of nucleated cells from those without a nucleus or debris, and live cells from dead or dying ones.



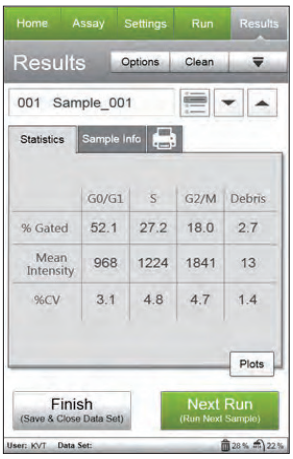
Anticipated Staining Pattern and Cell Size Index

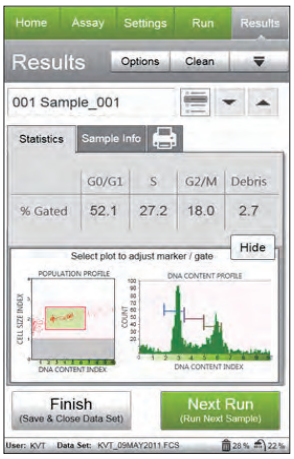
	Debris and non-nucleated cells	Viable Cells	Dying Cells	Dead Cells
Cell Size Index	Low	High	Med to High	High
Nucleated Cell Stain	Neg	High	High	High
Viability Stain	Neg	Neg	Med	High

Figure. 2.
Workflow (upper panel) and Principle (lower panel) for Muse™ Count & Viability Assay.

The assay utilizes a proprietary mix of two fluorescent DNA intercalating dyes to provide information on total cell concentration and viability (lower panel). One membrane permeable dye stains all cells with nuclei, allowing for the distinction of cellular debris from cells without a nucleus. The second dye only stains cells whose membranes have been compromised. Dying and dead cells stain with both dyes, but dying cells have lower fluorescence intensity than do dead cells.

▶ Cell Cycle Assay





Muse™ Cell Cycle Kit

Measure G0/G1, S, and G2/M phase distributions.

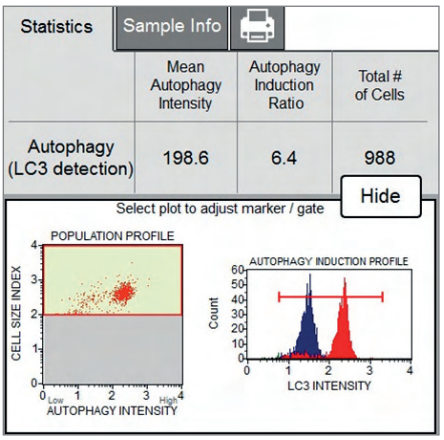
The Muse® Cell Cycle Assay uses the nuclear DNA stain propidium iodide (PI) to discriminate cells at different stages of the cell cycle, which differ in DNA content. Simply fix in ethanol and incubate with Muse® Cell Cycle Reagent for 30 minutes. After the samples are analyzed on the Muse® instrument, the percentages of populations are automatically displayed, along with a histogram with three markers to demarcate the G0/G1, S, and G2/M cell cycle phas

▶ Muse® Autophagy LC3-Antibody Based Assay

This kit enables quantitative analysis of autophagy using an anti-LC3 mouse* monoclonal antibody conjugated to Alexa Fluor®555, used to measure and track the levels of cytosolic and autophagosome-associated LC3. Also included is a selective permeabilization buffer, which discriminates cytosolic LC3 from autophagic LC3 by extracting the soluble cytosolic proteins while protecting autophagosome-associated LC3, thereby allowing its fluorescence to be measured by flow cytometry. Since autophagy is a constitutive cellular degradation process, the use of an autophagy detection reagent prevents the lysosomal degradation of LC3, allowing for quantification of its fluorescence.

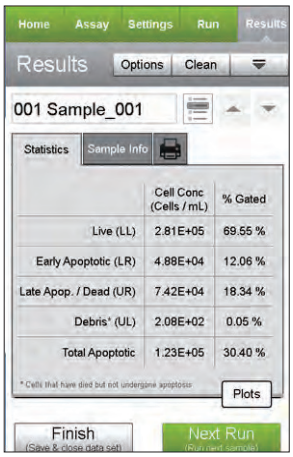
The following data outputs are shown:

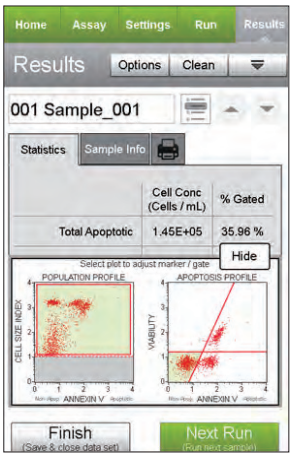
- Mean Autophagy Value (for both control and test samples)
- Autophagy Induction Ratio (test sample fluorescence relative to control)



■ Muse® Assays for Apoptosis Analysis

▶ Annexin V & Dead Cell Assay





Muse™ Annexin V and Dead Cell Kit

Differentiate live, early apoptotic, late apoptotic and dead cells.

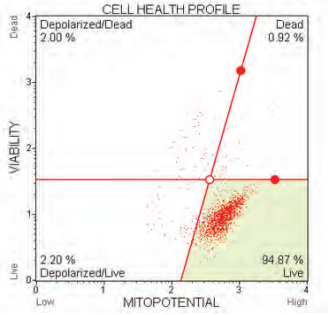
This versatile assay can be used to assess health of both adherent and suspension cells under multiple treatment conditions and generate dose-response data on cells treated with apoptosis inducers. The assay is based on the binding of Annexin V to phosphatidylserine (PS) on the surface of apoptotic cells. It uses a premixed reagent containing fluorescently labeled Annexin V and a dead cell marker (7-AAD). Early in the apoptotic pathway, molecules of PS are translocated to the outer surface of the cell membrane where Annexin V can readily bind to them with high affinity. Late-stage apoptotic cells show loss of membrane integrity and uptake of membrane-impermeant 7-AAD.

▶ MitoPotential Assay

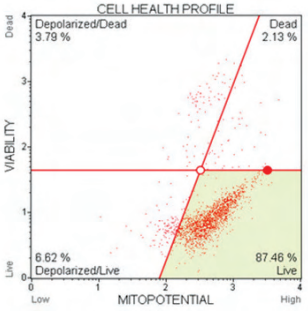
Differentiate live cells with intact & depolarized mitochondrial membrane and dead cells with intact & depolarized mitochondrial membrane.

Mitochondrial membrane potential changes have been implicated in apoptosis, necrotic cell death and caspase-independent cell death. Depolarization of the inner mitochondrial membrane potential is a reliable indicator of mitochondrial dysfunction and cellular health. This assay provides early and sensitive detection of cell health perturbation, enabling detection of mitochondrial depolarization under multiple treatment conditions in multiple cell types. The Muse® MitoPotential Assay uses the MitoPotential Reagent, a cationic, lipophilic dye, to detect changes the mitochondrial membrane potential, and 7-AAD as an indicator of cell death.

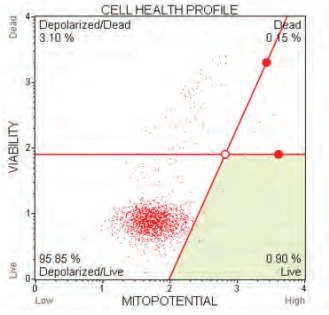
Jurkat Cells - untreated (A)



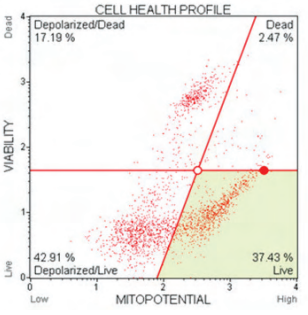
HeLa Cells- untreated (B)



Jurkat Cells- treated with CCCP (C)



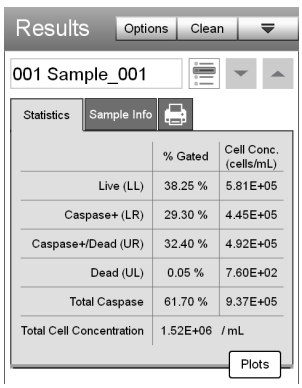
HeLa Cells- treated with Staurosporine (D)

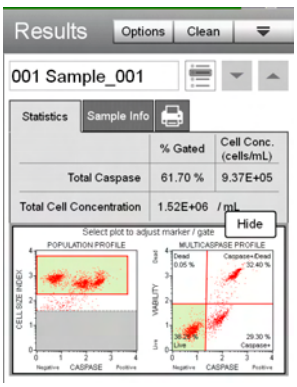


Impact of apoptosis-inducing compounds on Jurkat cells (suspension line) and HeLa cells (adherent line) using the Muse® MitoPotential Assay. Dot plots show untreated cells (A and B) and cells treated with CCCP (C) and Staurosporine (D).

▶ MultiCaspase Assay

This assay simultaneously determines the percentage and concentration of cells with caspase activity (detects following caspases 1, 3, 4, 5, 6, 7, 8 and 9), in combination with a dead cell dye. The Muse® MultiCaspase Assay uses a fluorogenic, derivatized VAD-peptide that can detect the activity of multiple caspases and a cell membrane impermeant dye, 7-AAD that provides information on cell membrane integrity. The VAD-peptide is derivatized with a fluorescent group and a fluoromethylketone irreversible caspase inhibitor moiety, generating a Fluorescent-Labeled Inhibitor of Caspases (FLICA). The peptide is membrane-permeable and non-cytotoxic. It binds to activated caspases with resulting fluorescent signal proportional to the number of active caspases in the cell. The dead cell marker, 7-AAD, is excluded from live (healthy) and caspase positive cells, but stains membrane-compromised, later-stage apoptotic and dead cells that show increased fluorescence in the viability axis.



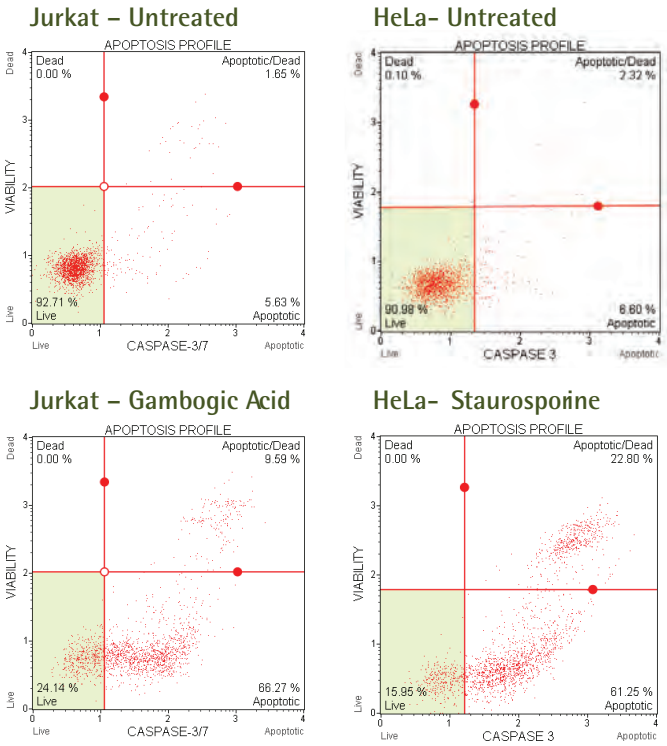


▶ Caspase-3/7 Assay

Differentiate live, mid-and late apoptotic and dead cells based on caspase-3/7 activity.

The Muse® Caspase-3/7 Assay determines the count and percentage of cells in various stages of apoptosis based on caspase 3/7 activity in combination with a dead cell dye. The Muse® Caspase-3/7 Assay uses the novel, fluorogenic Muse® Caspase-3/7 reagent for detecting caspase-3/7 activity and cell death dye, 7-AAD, that provides information on membrane integrity. The cell membrane-permeable Muse® Caspase-3/7 reagent contains a DNA-binding dye that is linked to a DEVD peptide substrate. While still conjugated to DEVD, the dye is unable to bind DNA. Cleavage by active caspase-3/7 in the cell results in release of the dye, translocation to the nucleus, binding of the dye to DNA and high fluorescence. The dead cell marker, 7-AAD, is excluded from live (healthy) and early apoptotic cells, but enters membrane-compromised, later-stage apoptotic and dead cells.

Impact of apoptosis-inducing compounds on HeLa cells and Jurkat cells analyzed using the Muse® Caspase-3/7 Assay.



■ Muse® Assays for Cell Signaling Analysis

Activation Dual Detection Kits are simple and precise, assays for study cell signaling pathways. Each kit include a pair of carefully optimized antibodies that bind to the same protein: one to detect total protein expression and another to detect the phosphorylated form of the same target. By using two-parameter analysis, the Muse® instrument delivers target-specific detection of phosphorylation while eliminating false positives and enhancing the signal-to-noise ratio. These kits also contain all the necessary fixation, permeabilization, and assay buffers to provide complete solutions for signaling analysis.

Data generated include:

- Percentage of inactivated cells
- Percentage of activated cells (via phosphorylation)
- Percentage of non-expressing cells

■ Muse® Assays for Immunology

Immunology deals with the functioning of the immune system in states of both health and disease and its physical, chemical and physiological characteristics and components. Immune cells interact with one another by a variety of signal molecules so that a coordinated response may be mounted against a pathogen or antigen. Flow cytometric analysis has always been a core technique for studying immune cells; now, the Muse® system brings the analyses you perform the most right to your bench top.

Existing Muse® Immunology Assays:

- Muse® Human CD4 T Cell Kit
- Muse® Human CD8 T Cell Kit
- Muse® Human B Cell Kit
- Muse® Human CD25 Lymphocyte Kit (for lymphocyte activation studies)
- Muse® Human CD69 Lymphocyte Kit(for lymphocyte activation studies)

Specifications

Features	Description
Ultra Compact Size - fits neatly in bench space	• Height : 8.69 inches (220.75 mm) • Width : 8.12 inches (206.37 mm) • Depth : 11.11 inches (282.15 mm)
3 Parameter, fluorescent based detection for maximum accuracy	• Detection of single cell voltage pulse for maximum accuracy, 2 colors plus forward scatter
Optics - Excitation	• Green Laser (532 nm)
Optics - Three detection channels	• Yellow fluorescence is detected within 28 nm bandwidth centered at 576 nm • Red fluorescence is detected within 30 nm bandwidth centered at 680 nm • Forward scattering signal is detected at laser wavelength
Fluidics	• Rectangular (1.5 mm x 0.8 mm) microcapillary with 100 um round bore
Highly intuitive software interface and sensitive touch screen	• Intuitive and guided menus to generate data output designed for each experimental application • Touch screen provides ease-of-use operation • Data Storage Capacity: 10GB
Dedicated software programs included	• Fully optimized software modules for count & viability, apoptosis and cell cycle for complete accuracy and intuitive analysis
Sample format	• Single loader. • Cell volume and number of events to acquire can be specified
Minimal sample volume	• Minimum/absolute sample volume needed: 200 µL (sample volume acquired depends on cell concentration) • Sample dead volume: 50 µL
User-specified input cell numbers	• User defined; cell concentrations of 10,000-500,000 cells/mL
Rapid sample processing time	• < 2 minutes per sample
Suitable for multiple cell types	• Homogeneous or heterogeneous cells, suspension or adherent • Primary cells or cell lines
Recommended Cell Size	• Size Range: 2-60 microns in diameter (not applicable for bacteria)
Data Handling	• Data outputs analyzed on system, and with USB export of graphs, CSV files, and raw data files for exported analysis

Ordering Information

Description	Cat. No.
Instrument and Accessories	
Muse® Cell Analyzer	0500-3115
Muse® Replacement Flow Cell	0500-3120
Instrument Cleaning Fluid (ICF) (100 mL)	4200-0140
Muse® System Check Kit	MCH100101
Additional Warranty	
1 year additional warranty, at time of purchase	0600-0360
1 year additional warranty, after purchase	0600-0365
2 year additional warranty, at time of purchase	0600-1780
2 year additional warranty, after purchase	0600-1785
Additional year of warranty, at purchase	0600-1790
Additional year of warranty, after purchase	0600-1795
Muse® Assays	
Cell Health	
Muse® Count & Viability Kit (100 tests)	MCH100102
Muse® Count & Viability Reagent (200x)	MCH100104
Muse® Autophagy LC3-Antibody Based Kit (50 tests)	MCH200109
Muse® RFP-LC3 Reporter Autophagy Assay Kit (100 tests)	MCH200110
Muse® Count & Viability Reagent (600 tests)	MCH600103
Muse® Oxidative Stress Assay (100 tests)	MCH100111
Muse® Nitric Oxide Assay (100 tests)	MCH100112
Muse® Ki67 Proliferation Assay (100 tests)	MCH100114
Muse® Cell Cycle Kit (100 tests)	MCH100106
Muse® Cell Dispersal Reagent (100 tests)	MCH100107

Description	Cat. No.
Apoptosis	
Muse® Annexin V & Dead Cell Kit (100 tests)	MCH100105
Muse® Caspase-3/7 Kit (100 tests)	MCH100108
Muse® MultiCaspase Kit (100 tests)	MCH100109
Muse® MitoPotential Kit (100 tests)	MCH100110
Cell Signaling	
Muse® H2A.X Activation Dual Detection Kit (50 tests)	MCH200101
Muse® EGFR-RTK Activation Dual Detection Kit (50 tests)	MCH200102
Muse® PI3K Activation Dual Detection Kit (50 tests)	MCH200103
Muse® MAPK Activation Dual Detection Kit (50 tests)	MCH200104
Muse® Bcl-2 Activation Dual Detection Kit (50 tests)	MCH200105
Muse® Multi-Color DNA Damage Kit (50 tests)	MCH200107
Muse® PI3K/MAPK Dual Pathway Activation Kit (50 tests)	MCH200108
Immunology	
Muse® Human CD8 T Cell Kit (100 tests)	MIM100102
Muse® Human CD4 T Cell Kit (100 tests)	MIM100101
Muse® Human B Cell Kit (100 tests)	MIM100103
Muse® Human CD25 Lymphocyte Kit (100 tests)	MIM100104
Muse® Human CD69 Lymphocyte Kit (100 tests)	MIM100105

To learn more, please visit "www.merckmillipore.com/muse"

Cell Counter

Scepter™ 2.0 Handheld Automated Cell Counter

Precision Cell Counting in a Portable, Personalized Format. The first and only device to bring consistency in cell counting right to the culture hood: the Scepter™ 2.0 cell counter. While other automated counters consume bench space and rely on object recognition software, manual focusing, and clumsy loading chambers, the Scepter™ cell counter provides true automation without the error that accompanies vision-based systems. With its microfabricated, precision-engineered sensor, the Scepter™ cell counter does all the work and delivers accurate and reliable cell counts in less than 30 seconds.

- Accurately count every cell in your sample
- Qualitatively determine cell health
- Count subpopulations in Peripheral Blood Mononuclear Cells (PBMC) preps
- Easily capture, analyze and manipulate data sets with Scepter™ Software Pro

Scepter™ Sensor Technology

Compatible with 60 µm and 40 µm sensors, the Scepter™ 2.0 cell counter can meet even more of your cell- and particle-counting needs. Use the 60 µm sensor for particles between 6 and 36 µm, and concentration 10,000–500,000 cells/mL. Use the 40 µm sensor for particles between 3 and 17 µm, and concentration 50,000–1,500,000 cells/mL.

- Precise volumes are drawn into the Scepter™ sensor.
- As cells flow through the aperture in the sensor, resistance increases. This increase in resistance causes a subsequent increase in voltage.
- Voltage changes are recorded as spikes with each passing cell.
- Spikes of the same size are bucketed into a histogram and counted. This histogram gives you quantitative data on cell morphology that can be used to examine the quality and health of your cell culture.

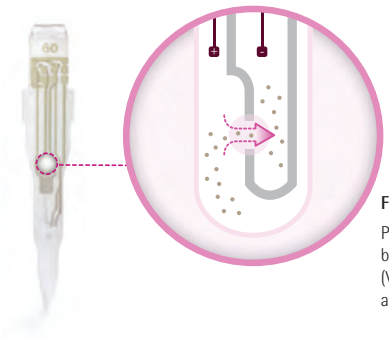


Figure 1.
Particles are detected by Ohm's Law V=IR (V=voltage, I=current, and R=resistance)

Cell Type	Measured size (µm)	40 µm sensor	60 µm sensor
2102 Ep	15-19		
454 beads			
A172	15		
A253	14-18		
A375	16		
A431	15-17		
A549			
Algae (various)	7-9		
B35	13-16		
B Cells	6-11		
C2C12	12		
C305	12-14		
C6	12-13		
CA46	10-12		
Caco-2	17		
CHO	14-17		
COS-1	12		
Cos-7	15		
D283	12		
Daudi	10-12		
DU-145	15-17		
Epithelia	14-15		
HCT-116	10		
HEK293	11-15		
HeLa	12-14		
HepG2	12		
HFF	18-20		
Hs27	14		
HT-1080	14-16		
HT-29	11		
HUH7-Hepatoma line			
Human ES Cells	9-12		
HUVEC	14-15		
IMR-32	12-14		
IMR-90	15		
Jurkat	13		
K562	22		
KB	14		
KG-1	10-13		
L6	14-16		
LNCaP	15-16		
Luminex® beads	5-6		
MCF7	15-17		
MDCK	13-15		

Cell Type	Measured size (µm)	40 µm sensor	60 µm sensor
Meg-01			
MG-63			
Mouse ES Cell			
Mesenchymal Stem Cell			
MRC-5			
NCI-H146			
NIH 3T3			
NTERA2, clone D1			
OK			
PBMCs			
PC12			
Primary Astrocytes			
Primary Neuronal Cell			
Raji			
Ramos			
Rat Dorsal Root Ganglion Cells			
Rat Whole Blood			
Red Blood Cells			
Rat Neural Stem Cell			
RAW 264.7			
RBL			
RIN-mF5			
SF9			
SH-SY5Y			
Sk-Br-3			
SK-MEL-28			
SK-N-MC			
SK-N-SH			
Splenocytes			
SW-480			
SW-620			
T84			
T98G			
TF-1			
U251			
U2OS			
U266			
U87-Human Glioblastoma cell line			
U937			
WI-38			
Y79			
Yeast- Pichia Pastoris			
Yeast- S.cerevisiae			

■ Recommended based on size
■ Merck Millipore Validated
■ Customer Validated

Table 1. Cell types validated with the Scepter™ cell counter and the recommended Scepter™ sensor.

Counting is Precise

You work too hard to lose data to erroneous cell counts. Be confident that your counts are right the first time. Scepter™ counting gives you the precision of the Coulter Counter™ at a price you can afford. Because the Scepter™ cell counter measures volume using the Coulter Principle, it can quantify cells based on size and will discriminate larger cells from smaller debris, unlike vision based techniques, which rely on object recognition software and cannot reliably detect small cells. The Scepter™ cell counter detects every cell and displays the population as a histogram of cell size distributions. From the histogram, count all the cells or use the gating function to count a chosen subpopulation. By monitoring changes in your histogram, you can gain insight into the health and quality of your cell culture from one experiment to the next.

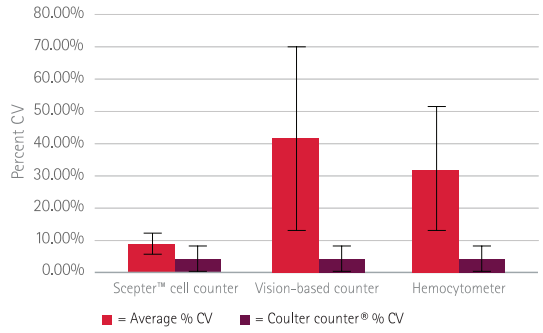


Figure 2.

The average percent coefficient of variation (CV) for each counting method shown was calculated using cell concentration measurements at 50,000 cells/mL samples of 19 different cell lines.

The Scepter™ cell counter is more precise than vision-based counting and hemocytometry, and approaches the precision of the Coulter Counter® standard (maroon bars). Error bars represent standard deviation.

	Format	Counting methods	Sample volume needed	Sample volume counted	Cells counted in a 100,000 cell/mL sample	Average % CV
Hemocytometer	Slide and microscope	Manual, vision-based	10 µL	.1 µL/square	10/square	41.8
Brand L	Benchtop	Automated vision-based system	10 µL	.4 µL	40	32.1
Scepter™ Cell Counter	Handheld	Impedance-based cell detection	100 µL	50 µL	5000	9.1

Counting is Reliable

We've tested dozens of cell types – including adherent, suspension, differentiated and progenitor cells – to validate the Scepter™ 2.0 cell counter's precision and operating range.

To obtain the data, cells of various types (adherent cancer cells (HeLa), suspension differentiated cells (U266, K562), suspension insect cells (SF9), and mesenchymal stem cells) were harvested and counted with a Scepter™ cell counter and 60 µm Scepter™ sensors. Counts are averages of 4 replicates, and error bars represent standard deviation.

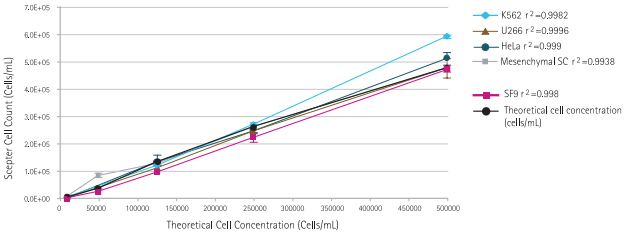


Figure 3. Scepter counting performs with high linearity (R2 > 0.993) across multiple, diverse cell lines, over a wide operating range. Shown here are cell concentration data for five representative lines out of 19 total cell lines tested.

Counting is Fast

Scepter™ counting is 7-10 times faster than hemocytometry, and faster than other automated counters, too. With Scepter™ technology, you'll save time and eliminate tedium.

The time required to perform cell counts using various methods was compared using a 500,000 cells/mL sample. Scepter™ counting (14 seconds on average, using a 60 µm sensor) is significantly faster than any of the other cell counting methods. Using 40 µm sensors, Scepter™ counts are complete within 25 seconds, on average (data not shown).

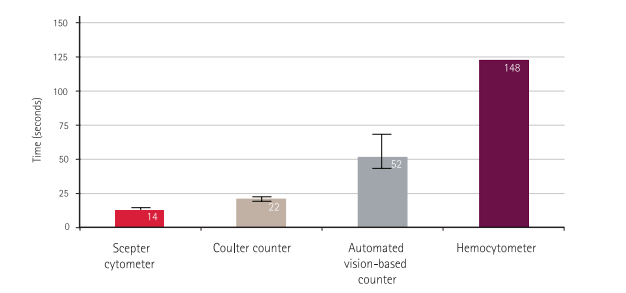
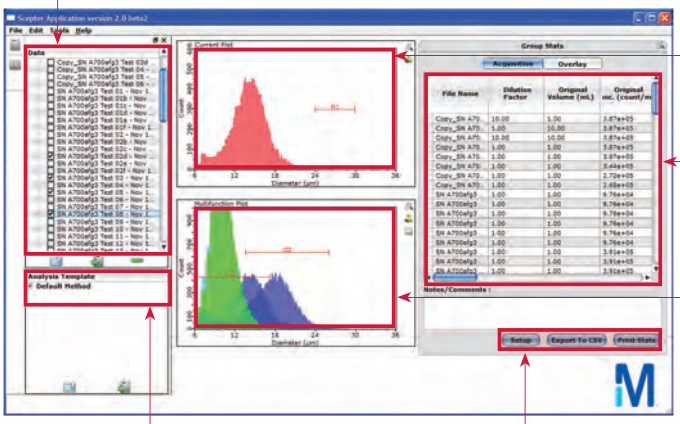


Figure 4. Cells (SF9, MCF7, and HEK293) were counted using the methods shown, and average time required to measure cell concentration was recorded. (Hemocytometry was performed only once per sample.)

Software

From simple counts to complex volume measurements used to assess cell health parameters, Scepter™ Software Pro provides an intuitive, intelligent platform to perform high-level cell analysis based on the size measurements captured with the Scepter™ cell counter.

A View of Scepter™ Software Pro



DATA : data files from your Scepter™ cell counter

CURRENT PLOT: working plot and data file

GROUP STATS: customizable statistics from your selected data files

MULTIFUNCTIONAL PLOT: multiple data sets/histogram overlays

REPORTS: export, print selected graphs/files, cut and paste

ANALYSIS TEMPLATES: saved gating parameters

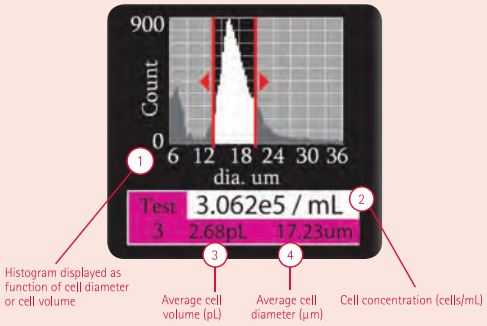
Using the Scepter™ Software Pro on your computer, you can:

- Compare several samples and data sets side by side using histogram overlay and multipara - metric tables
- Create and save gates to be used from one experiment to the next
- Create attractive graphical presentations and reports with your data

As easy to pipeting

Prepare the sample:

Start with a single-cell suspension, diluted to a total volume of 100 µL (recommended) in phosphate buffered saline (such as EmbryoMax® 1x DPBS) to 10,000-500,000 cells/mL (operating range for 60 µm sensor) in a 1.5 mL microcentrifuge tube.

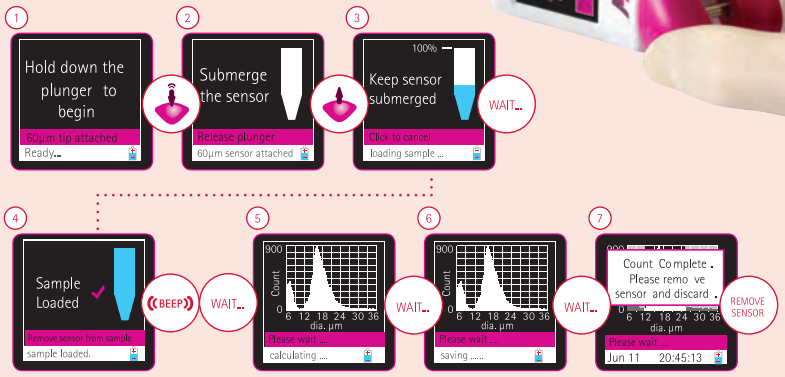


Perform cell count:

- Turn on the Scepter™ cytometer by pressing the toggle on the back of the instrument and wait for on-screen instructions to appear.
- When prompted, attach a sensor to the end of the Scepter™ unit with the electrode sensing panel facing toward the front of the instrument, and you'll see detailed istructions for each step of the counting process.
- Pipette once to draw sample into the sensor. 50 µL of your cell suspension is drawn into the microfabricated, precision-engineered channel embedded in the sensor. The cell sensing zone detects each cell drawn into the sensor and thus cell concentration is calculated.
- The sensing zone also measures cell sizes and cell volumes with sub-micron and sub-picoliter resolution, enabling the Scepter™ cytometer to display a histogram distribution of cell size or cell volume.

Discover how Scepter™ works, Step-by-Step:

Sample Preparation
▼
Cell Count
▼
Gating Process
▼
Exporting & Manipulating Data



Applications

Use the Scepter™ 2.0 Cell Counter to monitor multiple cell types in various applications! We continue to develop new applications for this versatile device; for the most recently developed application notes, please visit: **www.merckmillipore.com/scepter**

• Immuno-monitoring	• Cell Dealth	• Somatic Cell Counting	• Cell Cycle
• Bead Counting	• Human PBMC Counting	• Counting Yeast Cells	• Adipogenesis monitoring

Scepter™ 2.0 for Cell Health

Instantly gauge the health of your cell cultures without even leaving the culture hood.
Because the Scepter™ cell counter displays high-resolution histograms of entire cell populations, you can differentiate live cells from dead cells and debris by simply gating on the histogram peak corresponding to larger-diameter cells. No staining is required! The resulting calculation for % viable cells agrees with viability calculations obtained using flow cytometry (ViaCount® reagent) and Trypan blue staining/ hemocytometry (shown here with MDMA231 and NIH 3T3 cells).

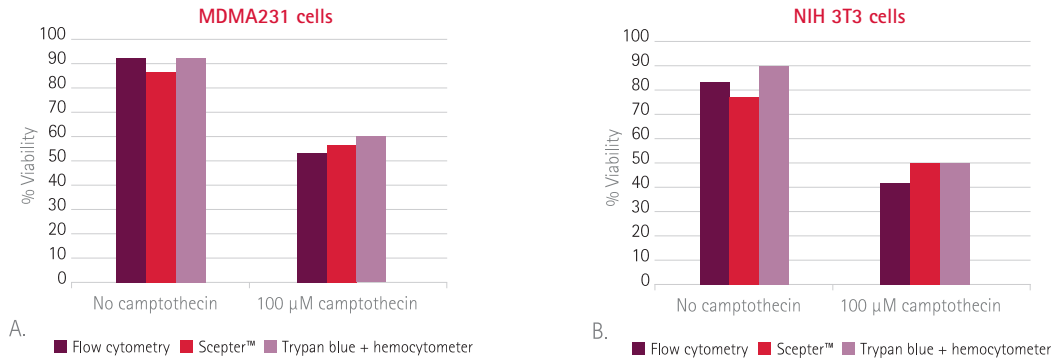


Figure 5. Rapid cell analysis using the Scepter™ 2.0 device provides reliable assessments of cell viability compared to flow cytometry (ViaCount® assay) and hemocytometry (using Trypan blue staining). MDMA231 cells (A) and NIH 3T3 cells (B) were treated with camptothecin 24 hours prior to analysis.

Scepter™ 2.0 for Counting Heterogeneous Cell Populations/ Immune Cell Analysis

Count blood cells and other cells with small diameters with the highest precision.

Biological samples such as primary isolates or cultured cells are often heterogeneous mixtures of cells that differ by type and/or function. Such differences in cellular attributes are most commonly determined by multicolor fluorescent antibody detection of cell type specific surface marker(s) using flow cytometry. Notably, in addition to variations in protein expression, many cell types and physiological states are also uniquely distinguishable on the basis of size alone. The ability to identify population subsets on the basis of phenotypic differences and further determine their relative frequencies (and concentrations) is critical to many aspects of research.

Distinguishing lymphocytes from monocytes in freshly isolated PBMCs.

The assessment of immune profiles of the various immune cell subsets can help identify molecular signatures that may facilitate research. The Scepter™ cell counter, when used in combination with Scepter™ Software Pro, provides a tool for rapid determination of lymphocyte and monocyte concentrations as well as the relative frequency of these cell types in PBMC isolates.

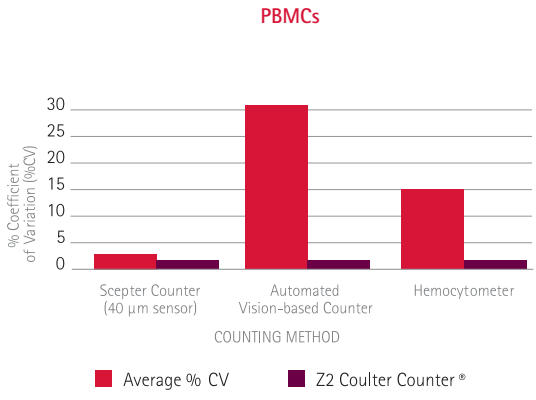
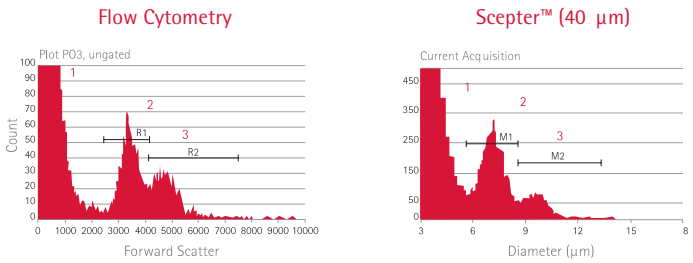


Figure 6. The Scepter™ 2.0 cell counter counts PBMCs with greater precision than other counting methods, as reflected by low coefficients of variation. % CVs were calculated using average cell counts of four replicate samples.



Test	Cell Fraction	Scepter™ [1]	Forward Scatter [2]	Staining [3]
1	Lymphocyte	58	65	63
	Monocyte	42	35	37
2	Lymphocyte	68	72	71
	Monocyte	32	28	29
3	Lymphocyte	66	69	71
	Monocyte	34	31	29

Figure 7.
Representative comparison of histogram plots for human PBMC samples

Acquired on the Scepter™ cell counter (diameter histogram on right) and guava easyCyte™ flow cytometry (forward scatter histogram on left) platforms. Analysis plots derived from both platforms demonstrate three distinct peaks corresponding to 1) dead cell/debris, 2) lymphocyte and 3) monocyte fractions. The difference in counts displayed (Y-axis) is due to differences in sample dilution between the guava easyCyte™ flow cytometer and the Scepter™ cell counter.

Table 2.
Lymphocyte and monocyte subset frequencies from three individual PBMC samples.

Aliquots from each sample were analyzed using the guava easyCyte™ flow cytometry and Scepter™ platforms. 1 - Values were derived from the diameter histogram plot. 2 - Values were derived from the forward scatter histogram plot based on total events measured on guava easyCyte™ flow cytometry platform. 3 - Staining frequencies derived as follows: % Lymphocytes = (% CD3+ T cells) + (%CD16/56+ NK cells) + (%CD19+ B cells); % Monocytes = % CD14+ cells

Assessing CD4+ T Cells Differentiated Towards Effector T Helper Cell Lineages

Murine CD4+ T cells can give rise to a variety of effector T, or "T helper", cell subsets depending on the nature of the immune response, and subsequently release a distinct subset of cytokines. Using the Scepter™ cell counter to rapidly assess size distributions of cellular populations provides a quick, simple method for tracking T cell differentiation.

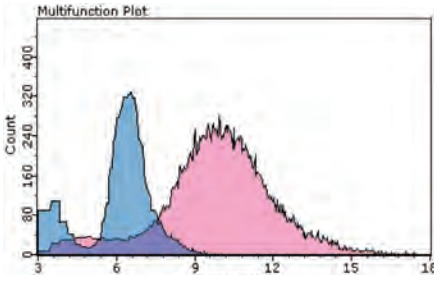


Figure 8. Th1, Th2, and Th17 cells expanded from approximately 6 to 10 µm when compared to the progenitor CD4+ T cell type. This expansion upon differentiation was clearly, accurately, and precisely measured using the Scepter™ cell counter. (Representative Th2 data shown here).

Immunomonitoring

In addition to variations in protein expression, many immune cell types and physiological states are also uniquely distinguishable on the basis of size alone. Using the Scepter™ 2.0 cell counter's sensitive size-discriminating capability, we demonstrated three examples of rapid, qualitative assessment of individual cell population frequencies in complex cell mixtures.

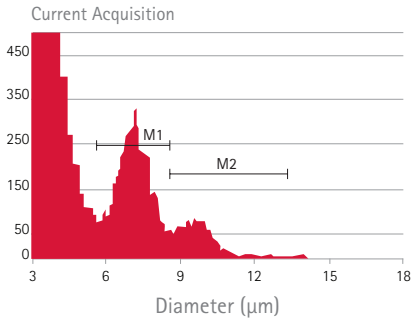


Figure 9. Scepter™ 2.0 counting was used to detect lymphocytes (right hand peak),monocytes (middle peak) and cell debris/dead cells (left hand peak) in human PBMC samples.

Scepter™ 2.0 for Specialized Applications

Counting Yeast Cells For Brewing And Wine Industries

Yeast cells are critical to the fermentation process for beer and wine production. There are several stages in the process at which analysis of the active yeast culture is critical. Here we show how the Scepter™ Cell Counter can be used to monitor the calculate yeast size and concentration.

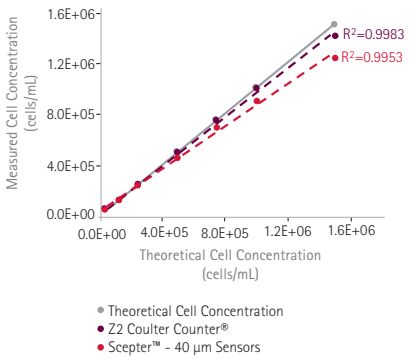


Figure 10. Comparing yeast cell counts from the Scepter™ cell counter and Z2 Coulter Counter®. Measured yeast cell concentrations are plotted against the theoretical concentrations. The solid gray line represents the theoretical values.

Adipogenesis Monitoring: Visualizing Differentiation Of Adipocytes

Adipocytes are derived from multipotent human mesenchymal stem cells (MSCs), providing researchers an ideal system for studying adipogenesis due to their multi-lineage differentiation potential. This study outlines a method for tracking adipogenic differentiation of ADSCs and 3T3-L1 cells using the Scepter™ Cell Counter, which can function as a reliable tool to track phenotypic change, in addition to generating highly precise cell counts.

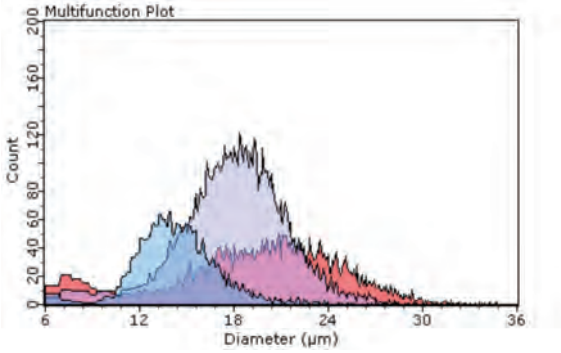


Figure 11. Preadipocytes (ADSCs) can be distinguished from differentiated adipocytes based on cell size by the Scepter™ cell counter. Cells gradually increased in size from 15 to 21 µm over the fourteen-day differentiation.

Ordering Information

Description	Qty	Cat. No.
Scepter™ 2.0 Handheld Automated Cell Counter		
with 40 µm Scepter™ Sensors (50 Pack)	1	PHCC20040
with 60 µm Scepter™ Sensors (50 Pack)	1	PHCC20060
Includes: Scepter™ Cell Counter, Downloadable Scepter™ Software, O-Rings, Scepterô Test Beads, Scepter™ USB Cable		
Scepter™ Sensors, 60 µm	50	PHCC60050
	500	PHCC60500
Scepter™ Sensors, 40 µm	50	PHCC40050
	500	PHCC40500
Universal Power Adapter	1	PHCCP0WER
Scepter™ O-Ring Kit, includes 2 O-rings and 1 filter cover	1	PHCC0CLIP
Scepter™ Test Beads	1	PHCCBEADS
Scepter™ USB Cable	1	PHCCCABLE
Additional Warranty		
1 year additional warranty to be ordered with the purchase of the Scepter		PHCC1YPWR
1 year additional warranty to be ordered before the end of the standard warranty		PHCC1YAWR

To learn more, please visit "www.merckmillipore.com/scepter"

Cell Culture Systems

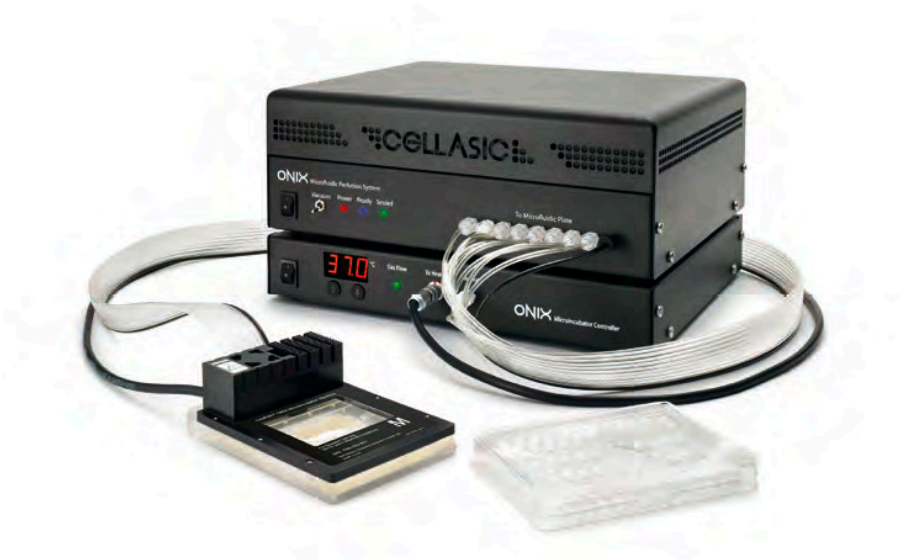
CellASIC™ ONIX Microfluidic Platform

The easy-to-use CellASIC™ ONIX Microfluidic Platform delivers precise control for live cell imaging experiments by facilitating long-term perfusion cell culture. The system integrates with your existing microscope system to enable dynamic time-lapse experiments never before possible. Cutting-edge microfluidics technology provides an improved cell culture microenvironment, exceptional quality for high magnification microscopy, and superior media switching capabilities.

- **Dynamic environmental control over live cells**
  Measure cell responses to pre-programmed perfusion, temperature, and gas environment changes. The CellASIC™ ONIX Microfluidic Platform automates all the requirements for live cell imaging, giving you the control you need to discover new science.

- **Bio-inspired microfluidic plates for optimized cell culture**
  CellASIC™ ONIX Microfluidic Plates are designed to optimize the health of specific cells during dynamic live cell experiments, including analyses requiring long-term culture, by creating a more in vivo-like environment. Application-specific plate designs give you the flexibility to probe the questions that interest you most and make it easy to image multiple microchambers in parallel.

- **Flexible, automated integration into virtually any protocol**
  Intuitive and easy-to-program CellASIC™ ONIX FG Software automates your entire customizable protocol, so you can spend more time exploring the countless experimental possibilities enabled by this single platform.



Technology

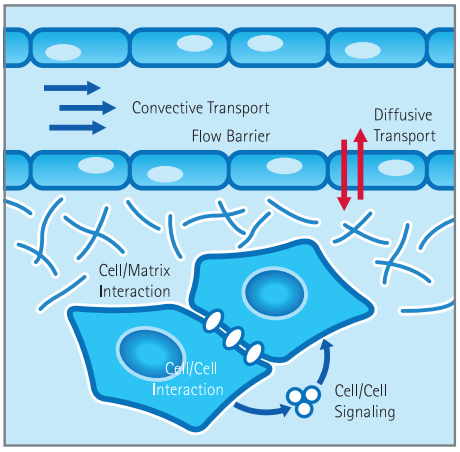
Delivering advanced control for live cell imaging experiments, the system integrates with your existing microscope to enable dynamic time-lapse experiments never before possible. Cutting-edge microfluidic technology provides an improved cell culture microenvironment, exceptional plate imaging quality for high magnification microscopy and superior media switching controls. An integrated Microincubator Controller maintains a temperature and gas environment directly on the microfluidic plate for long-term cell culture on any microscope stage.

"Think far beyond the limits of static cell culture."

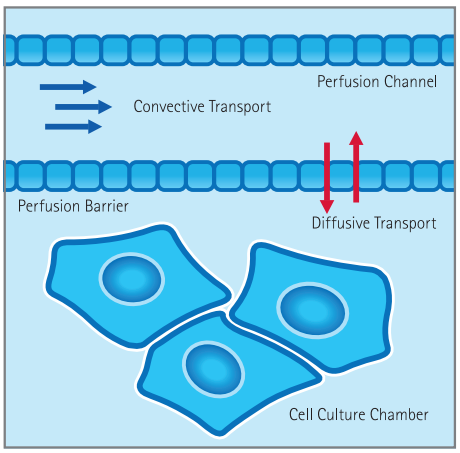
Biology is so much more than DMEM/FBS, 37 °C, 5% CO_2. Model your own creative designs and achieve true culture conditions with the new CellASIC™ ONIX Platform. With microfluidic precision, you can push the boundaries of your cell biology experiments in an in vivo-like environment. This new perfusion-based system enables you to program automated changes to culture conditions while maintaining optical access to cells through your microscope. You can even track individual cell responses over time by using a time-lapse-enabled microscope for truly dynamic cellular analysis.

What's missing from traditional cell culture and analysis?

Microfluidic perfusion mimics the *in vivo* cell environment



In vivo



Microfluidic

The analysis of living cells in vitro is critical to understanding basic biology, signaling pathways, drug effects, and disease models.
But despite dramatic advances in detection methods, which have provided excellent means to interrogate living cells, the technology for controlling the environment of living cells during that analysis has not advanced far beyond the culture dish.

Because the cellular microenvironment, or" niche," is as important as genetic factors for determining cell phenotype, a method for providing more accurate, dynamic control of living cells during experimental analysis can add a groundbreaking dimension to the science of cell biology.

The CellASIC™ ONIX Microfluidic Platform was specifically designed to provide the dynamic cellular microenvironment control that has been missing until now.

Just as nutrients and gases are transported through blood vessels, culture media components and gases are transported through perfusion channels of the CellASIC™ ONIX Microfluidic System. The perfusion barrier separating the cell culture from the channel (bottom) mimics the endothelial cell layer separating in vivo tissues from the blood (top).

CellASIC™ ONIX Microfluidic Platform

The CellASIC™ ONIX Microfluidic Platform overcomes the limitations encountered with traditional microfluidic setups. Whether your experiment demands cell culture in hypoxia or dynamic changes in temperature, the platform provides an easy-to-use, intuitive solution. The control system is connected to the microfluidic plate via a low-profile manifold, which enables setup on any inverted microscope. Gas and temperature controls connect directly to the cell culture chamber, eliminating the need for atmosphere and humidity control on your microscope. The uniquely designed platform components that enable live cell imaging include; Control System - Manifold – Microincubator

Control System

The control system contains its own, internal pressure/vacuum supply to enable flow control in any setting.
The system is the equivalent of 8 syringe pumps, a perfusion chamber apparatus, and a CO_2 incubator, at a fraction of the cost, with greatly improved quality and ease of use, and easily portable from one microscope to another. A low-profile manifold connects the control system to the plates, and is easy to adapt to any inverted microscope stage.

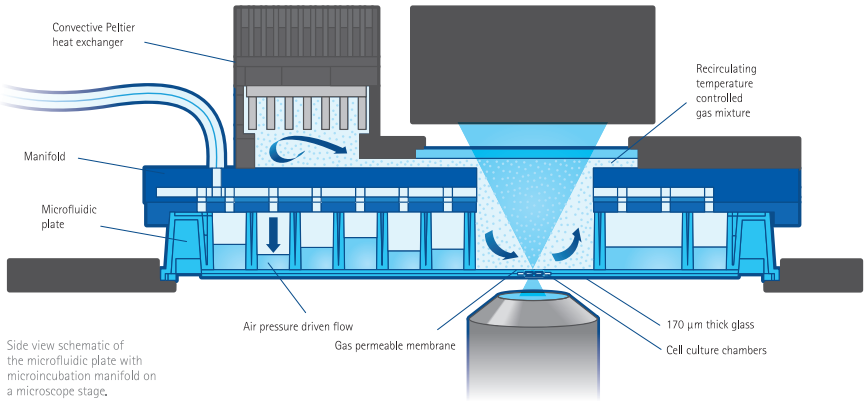
Manifold

A low-profile manifold connects the control system with the microfluidic plates. Various manifold layouts are available to address different plate formats and application types.

- Manifold forms an airtight seal to the top of the microfluidic plate, enabling pressure-driven flow of liquids to the cell chamber.
- No contact between the flow system and the biological solutions (on the plate), preventing contamination and lengthy clean-up.
- Low-profile manifold sits directly on top of the microfluidic plate, making it easy to adapt to any inverted microscope stage.
- Control apparatus is linked via air lines up to 2 meters away, saving precious microscope space.
- Plate/manifold assembly is held together by a vacuum mechanism, and takes only 1-2 seconds for" one-touch" manifold-to-plate sealing.

Microincubator

The CellASIC™ ONIX Microincubator Controller provides precise and rapid control of the gas and temperature environment.
The gas line feeds directly to the cell culture area via the innovative microincubation chamber created between the microfluidic plate and the manifold. The microincubation system maintains cells at 37°C, supplies 5% CO_2 to an enclosed 1 mL chamber above the cells, allows continuous medium perfusion, and prevents evaporation - all for up to three days on the microscope stage.



Side view schematic of the microfluidic plate with microincubation manifold on a microscope stage.

Figure 1.

Side view schematic of the microfluidic plate and the microincubation manifold on a microscope stage. The manifold and plate assembly fit into standard 96-well plate holders on any inverted microscope.
Since the manifold only contacts the microfluidic plate from the top, the bottom imaging surface (#1.5 thickness, 170 um, glass slide) is easily accessible by any objective lens. An imaging window cut through the manifold provides optical clarity.

CellASIC™ ONIX Microfluidic Plates

The CellASIC™ ONIX Microfluidic Plates deliver unprecedented control for live cell imaging. Our innovative, easy-to-use format is redefining the standard for microfluidics-based experimentation.

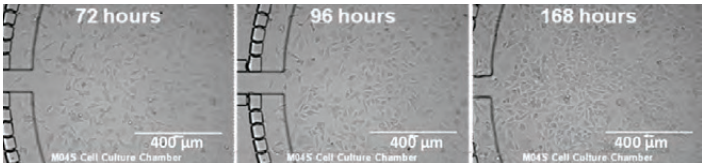
Pre-primed, disposable plates – When you receive the plates, they are"ready to go."
Pipette in your cells and sample solutions and begin your experiment.

Advantage

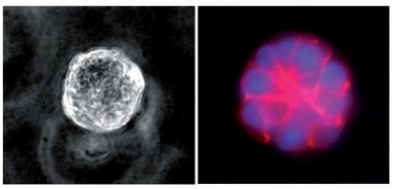
- Perform four independent experiments at once
- Compatible with any standard inverted microscope
- High resolution imaging though thin glass bottom
- Dynamic control over flow, gas and temperature
- Laminar flow for rapid solutions switching and stable gradient formation
- Perfusion barriers allow continuous mass transport without shear stress

▶ Optimized, bioinspired cell culture

Different cells need different environments. CellASIC™ ONIX Microfluidic Plates are designed to optimize the health of specific cells during dynamic live cell experiments, including analyses requiring long-term culture.
Various application-specific plate designs give you the flexibility to probe the questions that interest you most.



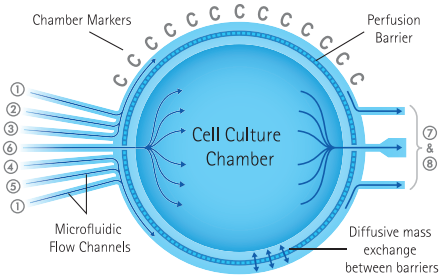
Healthy long-term cultures outside the incubator. NIH 3T3 cells were cultured in the CellASIC™ ONIX Microfluidic System (M04S plate) with continuous perfusion and monitored using bright field microscopy for 168 hours.



Robust three-dimensional cell cultures. MCF10A mammary epithelial cells were suspended in Matrigel® substrate and cultured with continuous perfusion for five days using the CellASIC™ ONIX Microfluidic System (M04L plate). Cells were stained for actin (red) and nuclei (blue). Brightfield and fluorescent images were acquired at 40X magnification.

▶ Automated integration into virtually any protocol

You're just minutes away from acquiring data using"load-and-go"CellASIC™ ONIX Microfluidic Plates. Intuitive and easy-to-program CellASIC™ ONIX FG Software automates your entire customizable protocol, so you can spend more time exploring the countless experimental possibilities enabled by this single platform.



1 Inlet for gravity driven continuous perfusion
2-5 Independent flow inlets for pressure driven flow
6 Inlet for cell loading
7-8 Outlets to waste wells

Follow these simple steps

1. **Load cells.** Prepare the microfluidic plate: Aspirate PBS from cell inlet well 6 and add 10 uL of desired cell suspension into the microfluidic plate. Cells will load automatically through capillary-driven cell loading.
2. **Load media or reagents.** Pipette reagents and media that will be used during your perfusion protocol into the four solution inlets (wells 2-5).
3. **Seal plate to manifold.** Seal plate to manifold by aligning the plate onto the manifold and turning on the vacuum switch on the CellASIC™ ONIX Microfluidic Platform. The plate is sealed when the green "sealed" light is lit
4. **Place assembly on microscope.** Seal plate to manifold by aligning the plate onto the manifold and turning on the vacuum switch on the CellASIC™ ONIX Microfluidic Platform. The plate is sealed when the green "sealed" light is lit.
5. **Create protocol using software.** Use the CellASIC™ ONIX FG Software's intuitive interface to program and monitor your experiment from one single view screen.
6. **Run protocol, perform live cell imaging..**

▶ Stable gradient for chemotaxis/migration assays

In order to create a quantitatively defined diffusion gradient that is stable enough for long-term, live cell imaging over the course of days, we developed a microfluidic gradient plate. This plate, designed for use on the CellASIC™ ONIX Microfluidic Platform, enables precision-controlled chemoattractant diffusion across perfusion barriers to create a spatial gradient in the culture area. On the other end of each culture area, there are flow outlets. Together, the perfusion inlets and outlets form a continuousflow "infinite source/sink" that maintains a stable concentration gradient profile for days. The flexible format of the plate enables changes in gradient directionality, turning gradients on and off, and toggling between gradient and single solution exposure.

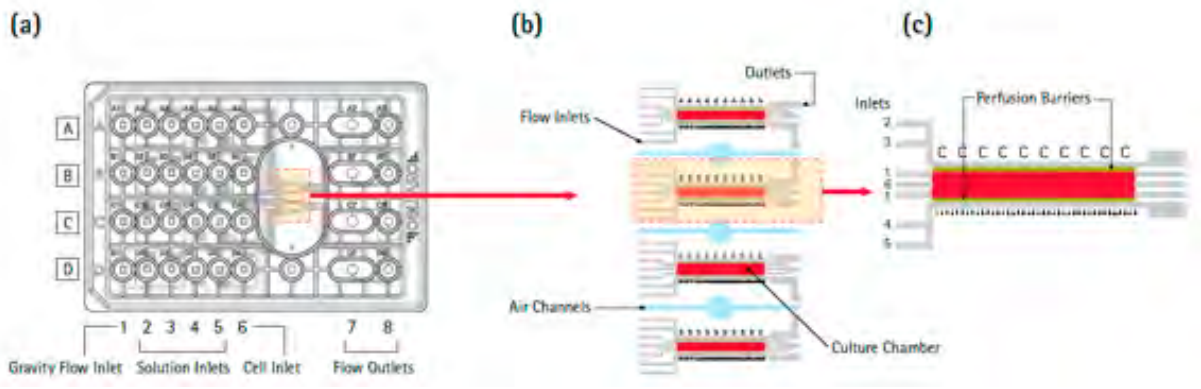


Figure 2.
(a) The CellASIC™ ONIX M04G Microfluidic Plate has 4 independent culture chambers (A-D), each with a gravity flow inlet (1), four solution inlets (2-5), a cell inlet (6), and two shared outlet wells (7 and 8). Each row of wells (A-D) addresses the corresponding culture chamber. (b) All four culture chambers are located under a single imaging window to minimize travel distance for high magnification phase objectives. (c) The chamber is bound by perfusion barriers on the top and bottom edges to separate the chamber from flow channels. Inlet wells 2 and 3 flow media into the upper channel, while 4 and 5 flow media through the lower channel. Gradients are established by simultaneously flowing media of different compositions through the upper and lower channels. Due to continuous perfusion, a stable gradient can be maintained for extended periods (> 2 days).

Software

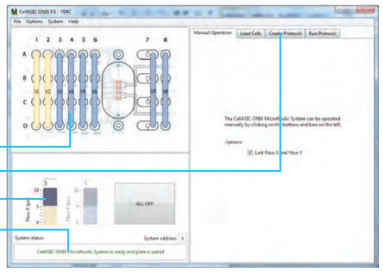
Intuitive and flexible software controls the CellASIC™ ONIX Microfluidic System to run microfluidic experiment plates. The push button controls let you start your experiment with a few quick mouse clicks. The CellASIC™ ONIX FG software also enables programmable, "hands-free" flow sequencing using application specific wizards or using the simple text-based command language. This enables experiments where timing of flow switching is critical, as well as long time-lapse experiments when constant user supervision is not feasible.

CellASIC™ ONIX FG Software Key Features:

- Intuitive control of pressure driven flows
- Plate-specific wizards simplify flow programming
- Flexible user-defined flow programs
- USB connection to PC

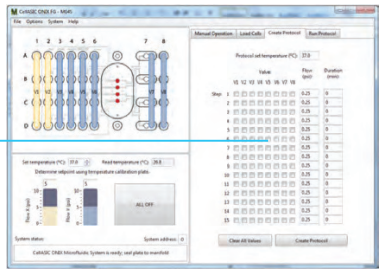
Manual Operation
Click with your mouse to control inputs, outputs, gas and temperature in real time.

Valve on/off Buttons
Function Tabs
Regulator Setpoints
Status Bar

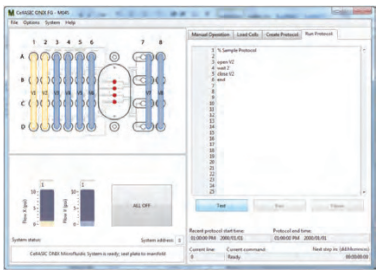


Create Protocol
An easy Wizard helps you set up an automated protocol for pre-programmed, walk-away perfusion changes over minutes, hours or days.

Protocol Wizard:
Click the valve and enter the time and flow rate for each step. Design 5 steps or 15–it's all under your control.



Run Protocol
On this tab, you can save, change or add steps to the protocol you created using the" Create Protocol" Wizard.



Applications

Popular applications of the CellASIC™ ONIX Platform

What you've always imagined can now be reality, using the CellASIC™ ONIX Platform to design dynamic cell biology experiments. It's been demonstrated by our own scientists and loyal customers. The applications listed below are just a few of the exciting experiments you can perform with unprecedented precision.

• Long Term Cell Culture	• Live Cell Imaging and Hypoxic Culture	• Imaging
• Interactions	• Neural Stem Cells	• Chemotaxis/Migration
• Cell Response Over Time	• 3D Cell Culture	• Protein Localization or Translocation
• Drug Dose/Response	• Hypoxic Conditions to Mimic Tumor Microenvironments	• Other Applications
• Bacteria Single Cell Imaging	• Yeast Single Cell Imaging	

■ Long-Term Cell Culture and Gene Expression Analysis

Microfluidic perfusion enables long-term cell culture, precise microenvironment control and gene expression analysis

The CellASIC™ ONIX Microfluidic System, in conjunction with the CellASIC™ ONIX Microfluidic Plate, provides perfusion-based microenvironment control for longterm, high quality, live cell microscopy (Figure 3). The microfluidic chamber recreates the physiologic mass transport condition for optimized cell health. Four upstream fluidic channels allow controlled exposure of the cells to different solutions during live imaging. The plate can also be cultured in a standard incubator using a dedicated gravity driven flow channel. The cells are in contact with a #1.5 thickness (170 um) optical glass surface, enabling high quality imaging using an inverted microscope. An integrated microincubator system delivers temperature and gas control to the microfluidic chambers.

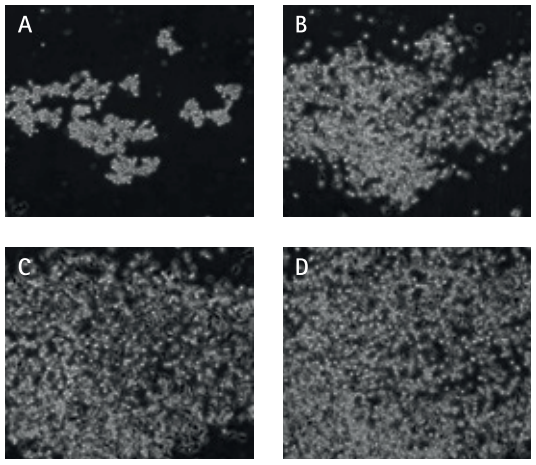


Figure 3.

Growth of MDA-MB-231 cells in the microfluidic chamber with continuous perfusion after (A) 1 hour, (B) 1 day, (C) 2 days, and (D) 3 days. Images were acquired with a 4X objective lens.

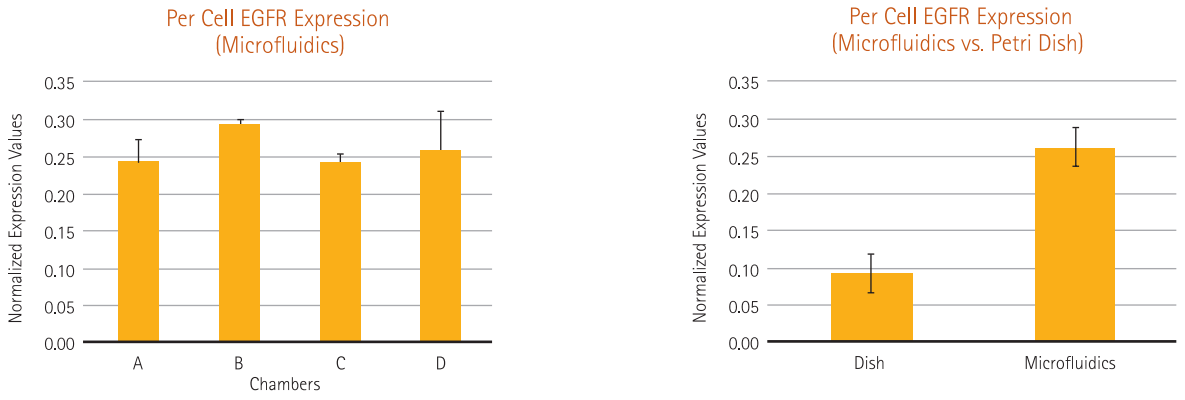


Figure 4.

(Left) Per-cell EGFR expression normalized to GAPDH expression and total number of cells per chamber. (Right) Comparison of per-cell EGFR expression of MCF-10A cells cultured in the CellASIC™ ONIX System (M04S plate) vs. in standard Petri dish.

Host Pathogen

Long term, live cell imaging of host-pathogen interactions using the CellASIC™ ONIX System

The CellASIC™ ONIX Microfluidic System is well suited for host-pathogen studies by providing a stable, long term culture environment for host cells (including primary cells) with controlled pathogen exposure. The continuous perfusion format and the ability to switch media solutions enable wash-out of pathogens from the chamber and subsequent monitoring of host cell response over many days. The enclosed small volume of the culture chamber also provides practical advantages for working with infectious agents during live cell imaging. The design of the microfluidic plates enables the dynamics of infection to be tracked using live cell imaging on standard inverted microscopes.

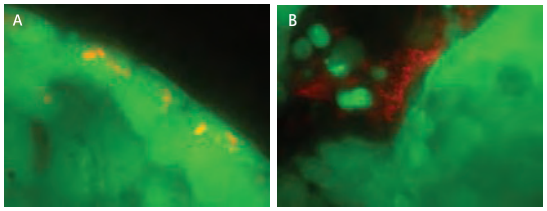


Figure 5.

Images of (A) an invasive strain and (B) a non-invasive strain of E. coli after exposure to human HT-29 cells, washout, and perfusion culture. Bacteria expressed mCherry, and HT-29 cells stained with Calcein AM. Panel (A) was acquired with a 100X objective lens, and panel (B) was acquired with a 60X objective lens.

Live Cell Imaging and Hypoxic Culture

Microincubator for Long-Term, Live Cell Microscopy and Hypoxic Culture

The CellASIC™ ONIX Microincubator is a breakthrough system enabling precise environment control for long-term microfluidic cell culture with clean integration with existing live cell imaging microscopes. In previous work, it was demonstrated that the CellASIC™ ONIX microfluidic culture chambers provided unique advantages for cell biology experiments. However, the reliance on existing microscope temperature/environment control solutions limited accessibility of the microfluidic platform, while also proving inadequate for many applications. Here, we validated the performance of the CellASIC™ ONIX Microincubator to deliver precise and stable temperature and gas control for multi-day microscopy applications and for culturing cells under precisely controlled gas compositions. The combination of microfluidic perfusion culture, environment control, and optical quality makes the CellASIC™ ONIX Microincubator the ideal platform for long-term live cell imaging experiments that require precise gas environment control, such as hypoxia.
The continued adoption of this technology will lead to further insights into physiologically predictive in vitro cell models and improved data relevance.

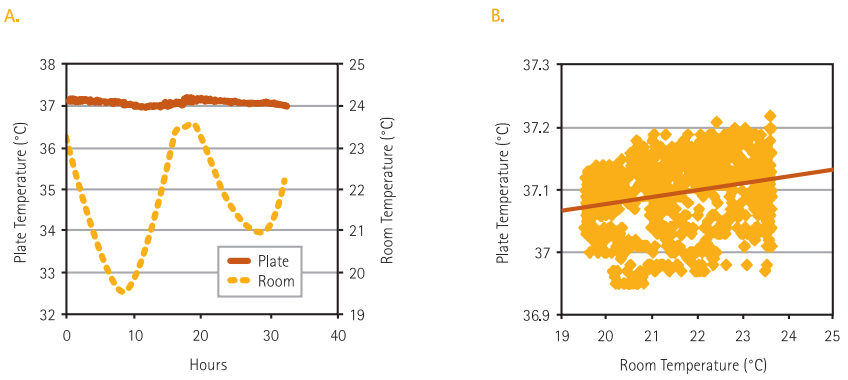


Figure 6.

Temperature stability of the CellASIC™ ONIX Microincubator during live-cell imaging.
(A) Time course of microfluidic chamber temperature ("plate") over 36 hours on the microscope stage, showing about 0.2 °C fluctuation.
(B) Plate temperature plotted against room temperature.

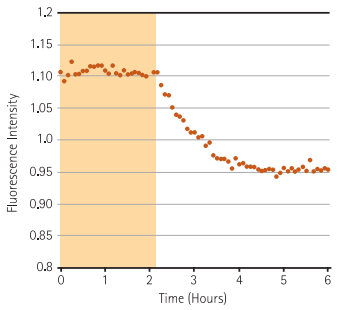


Figure 7.

Oxygen-sensitive dye RTDP indicated that the gaseous microenvironment was exchanged from hypoxic to normoxic conditions in around 2 hours.

■ Rat Neural Stem Cells (NSCs)

ONIX Live Cell Imaging Platform for Neural Stem Cell Microenvironment Control

Neural stem cells (NSCs) are sensitive to microenvironmental cues, including cell-cell contact, cell-ECM interaction, nutrient and waste transport, as well as environmental oxygen composition. However, how these parameters in the microenvironment affect the stem cell's morphology, proliferation, and differentiation remains an open area for research.

The CellASIC™ ONIX Microfluidic Platform offers comprehensive cellular microenvironmental control for cell culture studies. The ability to control multiple parameters has been integrated into the CellASIC™ ONIX Microfluidic Platform to enhance the cellular microenvironment for NSC culture. To modulate the degree of cell-cell contact, the system can be set up to seed cells at varying density, leading to varying spatial distribution.

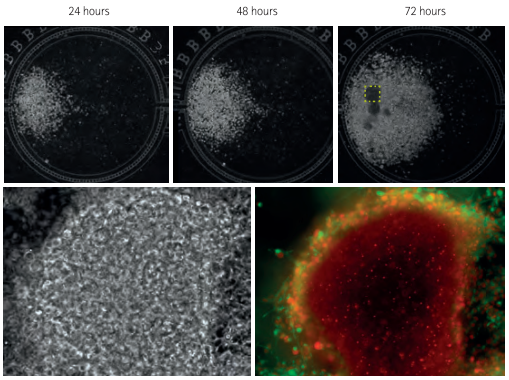


Figure 8.
High density seeding of NSCs in a microfluidic chamber under conditions of mild hypoxia causes formation of neurospheres (top row, inset box at 72 hours). Neurospheres were also imaged at higher magnification (20X, bottom left). When stained for nestin and Sox2 (bottom right), only the outer layers of the neurospheres showed both nestin (green) and Sox2 (red) expression; the core displayed bright spots of Sox2 expression. This pattern was possibly caused by neurosphere compression by the ceiling of the microfluidic chamber.

■ Key publications using the CellASIC™ ONIX Microfluidic Platform

Wei P., Wong W., Park J., Corcoran E., Peisajovich S., Onuffer J., Weiss A., Lim W. Bacterial virulence proteins as tools to rewire kinase pathways in yeast and immune cells. Nature, 2012 August 16; 488(7411):384-8.

Bermejo C, Haerizadeh F, Takanaga H, Chermak D, Frommer W. Optical sensors for measuring dynamic changes of cytosolic metabolite levels in yeast. Nature Protocols. 2011 October 27 6;1806-1817.

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Eser U, Falleur-Fettig M, Johnson A, Skotheim J. Commitment to a cellular transition precedes genome-wide transcriptional change. Molecular Cell. 2011 Aug 19 4;43:515-527.

Tamura N, Oku M, Sakai Y. Atg8 regulates vacuolar membrane dynamics in a lipidation-independent manner in Pichia pastoris. J Cell Sci. 2010 Dec 1;123(Pt 23):4107-16.

Bermejo C, Haerizadeh F, Takanaga H, Chermak D, Frommer WB. Dynamic analysis of cytosolic glucose and ATP levels in yeast with optical sensors. Biochem J. 2010 Sep 20.

Dechant R, Binda M, Lee SS, Pelet S, Winderickx J, Peter M. Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. EMBO J. 2010 Aug 4;29(15):2515-26.

Manzoni R, Montani F, Visintin C, Caudron F, Ciliberto A, Visintin R. Oscillations in Cdc14 release and sequestration reveal a circuit underlying mitotic exit. J Cell Biol. 2010 Jul 26: 209-22.

Furuya K, Niki H. The DNA damage checkpoint regulates a transition between yeast and hyphal growth in Schizosaccharomyces japonicus. Mol Cell Biol. 2010 Jun;30(12):2909-17.

Octavio LM, Gedeon K, Maheshri N. Epigenetic and conventional regulation is distributed among activators of FLO11 allowing tuning of population-level heterogeneity in its expression. PLoS Genet. 2009 Oct;5(10):e1000673.

Thorn K. Spinning-disc confocal microscopy of yeast. Methods of Enzymology, vol 470, 2010, 581-602.

Lee PJ, Gaige TA, Hung PJ. Dynamic cell culture: a microfluidic function generator for live cell microscopy. Lab Chip. 2009 Jan 7;9(1): 164-6.

Specifications

CellASIC™ ONIX Microfluidic Platform (EV262 Microfluidic System and MIC230 Microincubator Controller)	
Microscope Compatibility	Inverted microscope
Microscopy Techniques	Fluorescence, Brightfield, Phase Contrast, Confocal, TIRF, and DIC Microscopy
Imaging Substrate	#1.5 glass coverslip
Microfluidic Plate Footprint	96-well plate footprint
Number of Chambers	4 microfluidic cell culture chambers (in parallel)
Culture Time with CellASIC™ ONIX Microfluidic Platform	1-3 days continuous
Cell Suspension Volume	5-10 µL (M04 CellASIC™ ONIX Microfluidic Plates), 50 µl (B04/Y04/C04 CellASIC™ ONIX Microfluidic Plates)
Number of Pressure Inputs	8 inputs
Output Pressure Range	0-10±0.25 psi (0-70±1.7 kPa)
Optical Transparency	Optically clear manifold and microfluidic plates
Optional Premixed Gas Input	Works with clean, dry, premixed gas containing air, CO2, nitrogen and oxygen up to 25% regulated to between 45-55 psi (310-379 kPa).
Temperature Control Range	Room temp. to 40° C
Rise Time (25° C to 37° C)	<10 minutes
Cooling Time (37° C to 25° C)	<15 minutes
Gas Consumption	3 mL/min, ±0.5 mL/min
Dimensions	310 mm Wide x 257 mm Deep x 163 mm High

Cell types used with the CellASIC™ ONIX Microfluidic Platform	
Adherent Cells	HeLa, CHO Cell, NIH-3T3, MCF-7, MCF-10A, PC-3, HUVEC, PC-12, HL-60, HT-29, Neuron Cells (Hippocampal/Cortical), Cardiomyocytes
Non-Adherent Cells	Macrophages, Lymphocytes, T Cell, Bacteria (E. coli, B. subtillus, Cyanobacteria, M. smegmatis), Yeast (S. cerevisiae, S. pombe), Chlamydomonas
ECM Coating Substrates Used	Fibronectin, Collagen, Matrigel® substrate, Poly-D-lysine, Laminin, Hydrogels

Ordering Information

Description	Cat. No.
CellASIC™ ONIX System Components	
CellASIC™ ONIX Microfluidic System Package includes CellASIC™ ONIX Microfluidic Perfusion Controller, Manifold, Accessory Box, and CellASIC™ ONIX FG Software	EV262
CellASIC™ ONIX Microincubator Package for Temperature and Gas Control: Includes CellASIC™ ONIX Microincubator Controller, Microincubator Manifold, and Accessory Box	MIC230
CellASIC™ ONIX Tri-gas Mixer: Compressed Air, CO2, and Nitrogen Gas Mixer	GM230
Additional Warranty	
1 year additional warranty	2YRWAR
2 year additional warranty	SVC3YRWAR
3 year additional warranty	SVC4YRWAR

To learn more, please visit "www.merckmillipore.com/cellasic"

Description	Qty	Cat. No.
CellASIC™ ONIX Microfluidic Plates		
B04A Microfluidic Plate for Bacteria Cells (4 Chambers)	5/pk	B04A-03-5PK
C04A Microfluidic Plate for Chlamydomonas Cells (4 Chambers)	5/pk	C04A-01-5PK
M04G Microfluidic Gradient Plate for Mammalian Cells (i.e. migration, chemotaxis) (4 Chambers)	5/pk	M04G-02-5PK
M04L Microfluidic Open-top Plate for Mammalian Cells > 50 µm (4 Chambers)	5/pk	M04L-03-5PK
M04S Microfluidic Switching Plate for Mammalian Cells (4 Chambers)	5/pk	M04S-03-5PK
Y04C Microfluidic Plate for Haploid Yeast (cells 3.5 - 4.5 µm) (4 Chambers)	5/pk	Y04C-02-5PK
Y04D Microfluidic Plate for Diploid Yeast (cells 5-7 µm) (4 Chambers)	5/pk	Y04D-02-5PK
CellASIC™ ONIX Manifolds		
Manifold for use with CellASIC™ ONIX plates (without heating)	1/pk	F84-GL4
Manifold with recirculating heat exchanger, for use with CellASIC™ ONIX plates and Microincubator Controller	1/pk	F84-HG4
Manifold for use with CellASIC™ ONIX plates, for DIC imaging	1/pk	F84-DL3
Manifold for use with CellASIC™ ONIX plates, small for deep well plate holders	1/pk	F84-GL2

Luminex® Instruments

MAGPIX®
Luminex® 200™
FLEXMAP 3D®

Accelerate your research with Luminex instruments, Merck Millipore's MILLIPLEX MAP multiplex kits and bead washers. This complete solution gives you the power of Luminex® xMAP® technology for biomarker screening and protein analysis, and access to the largest portfolio of multiplex analytes available. As a Luminex partner, Merck Millipore is a prefered distributor of the entire Luminex portfolio of instruments, including LX 200™, FLEXMAP™ 3D and the MAGPIX® platforms, covering the full range of multiplexing capabilities.

- **Speed / High Throughput**
  Simultaneously measure the concentration of a large number of different analytes in a single sample, enabling you to do your work faster, gaining early and comprehensive data so critical to your work
- **Versatility**
  A single xMAP technology-based system can perform assays in several different formats, including nucleic acids and antigen-antibody binding, along with enzyme, receptor-ligand and other protein interactions
- **Flexibility**
  The technology can be customized for the user's specific needs based upon their analytes of interest
- **Accuracy**
  The technology generates real-time analysis and accurate quantification of the biological interactions
- **Reproducibility**
  High-volume production of xMAP microspheres within a single lot allows assay standardization that solid-phased flat arrays cannot provide
- **Low sample volume**
  With minimal hands-on time, you can screen more than 40 analytes in a single sample using as little as 25 µL
- **Magnetic bead-based format**
  Responds rapidly and efficiently to a magnetic field, enabling better and faster washing techniques, including high-throughput options

Luminex xMAP® Technology
The Power of Simultaneous Multi-Analyte Detection

The Luminex System is the combination of four core technologies:

xMAP® microspheres:

- xMAP® magnetic bead microspheres are a family of 80 fluorescently dyed 6.45 µm magnetic microspheres that act as both the identifier and the solid surface to build the assay.
- Traditional agarose microspheres are a family of 500 fluorescently dyed 5.6 µm polystyrene microspheres that act as both the identifier and the solid surface to build the assay.

Luminex® analyzer:

- Luminex 200™ and FLEXMAP 3D® systems: a flow cytometry-based instrument that integrates key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
- MAGPIX® system: a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency enabled by magnetic beads.

Software:

- XPONENT® software package for data acquisition.
- MILLIPLEX® Analyst 5.1 software package for sophisticated data analysis.

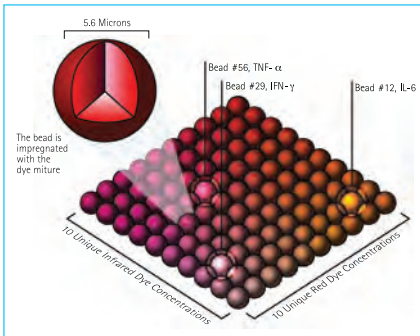
Bead-based immunoassays:

- MILLIPLEX® map multiplex assays consist of analyte-specific capture antibodies conjugated to xMAP® beads, enabling multivariate analysis of complex biological states, including metabolic disease, immunology, neurodegenerative disease, toxicity, cancer and more, using minimal sample volumes. MILLIPLEX® map assays are analytically validated for sensitivity, specificity, reproducibility and wide dynamic range.

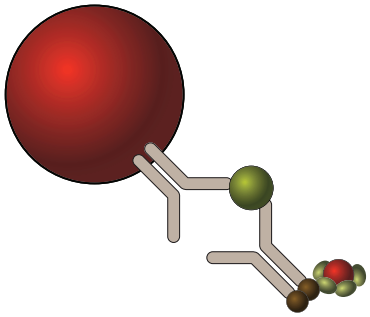
The rapidly growing knowledge base in drug discovery and protein research has placed increased pressure on researchers to quickly gain a greater understanding of protein-protein interactions, cell signaling pathways and markers of metabolic processes. Increasingly, this information is difficult or impractical to obtain using traditional "singleplex" protein detection methods, such as ELISA or Western blotting.

By enabling you to add multiple conjugated beads to each sample, Luminex's xMAP technology lets you obtain multiple results from each sample for fast, flexible, affordable and high throughput biological testing technology. What's more, the open- architecture xMAP technology enables multiplexing of many types of bioassays, reducing time, labor and costs over traditional methods.

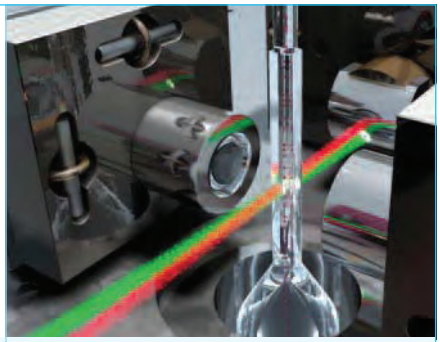
xMAP® Technology Process Flow



Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.



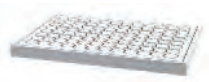
After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced. The reaction mixture is then incubated with Streptavidin PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.

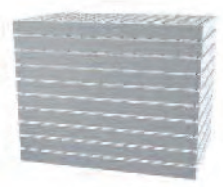


The microspheres are allowed to pass rapidly through a laser, which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.

▶ Comparison of the xMAP and ELISA Technologies

Side-by-sie comparison of Multiplexing vs. ELISAs
(study of 10 different analytes in 80 test samples)

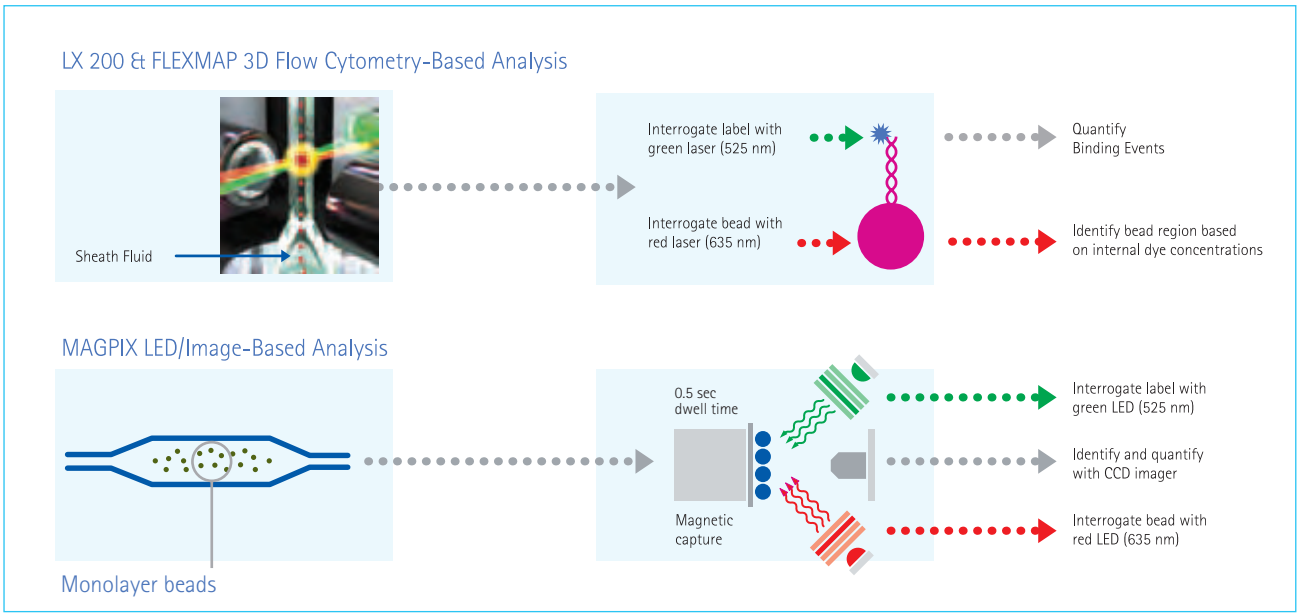




	xMAP Technology	ELISA
Number of plates required	1	10
Results per plate	800	80
Total sample used per panel	50 µL	4 ml
Internal controls possible?	YES	NO
Dynamic Range	1-10,000 pg/ml	10-2500 pg/ml
Lower limit of detection	~1 pg/ml	~1 pg/ml

■ MAGPIX xMAP Detection Comparison

MAGPIX takes advantage of a new lighting and detection mechanism to reduce costs and improve reliability. Unlike flow cytometry which leverages the physics of fluid flow to align beads single file through a cuvette, where they can be illuminated with higher cost lasers, MAGPIX flows beads with considerably less fluid volume into a chamber. There they are held in a monolayer with a magnet. LEDs are focused on the chamber to illuminate the beads. In flow cytometry, the fluorescence of the beads is measured with a Photo Multiplier Tube. MAGPIX, takes an image of the beads in the chamber with a CCD camera. Image processing algorithms are run to deliver the assay results.



Choose the Instrument That's Right for You

Specifications

Instrument	MAGPIX	Luminex 200	FLEXMAP 3D
Software	xPONENT 4.2	xPONENT 3.1	xPONENT 4.2
Optic	LED / CCD Camera	Lasers / APDs / PMTs	Lasers / APDs / PMTs
Hardware	Fluorescent Imager	Flow Cytometry based	Flow Cytometry based
Bead Compatibility	Magnetic	Magnetic and non-Magnetic	Magnetic and non-Magnetic
Multiplex Capacity	50	100 (80 for MagPlex)	500
Read Time	~ 60 mins / 96 well plate	~40 mins / 96well plate	~20 mins / 96well plate
Applications	Protein / Nucleic Acid	Protein / Nucleic Acid	Protein / Nucleic Acid
Dynamic Range	3.5 logs	3.5 logs	4.5 logs
Microtiter Plate	96 well	96 well	96 well & 384 well
Footprint including PC (linear bench space)	64.8 cm (24î)	80.0 cm (32î)	64.8 cm (24î)
Weight (Analyzer)	17.5 kg (38.5 lbs)	49 kg (113 lbs)	77.1 kg (170 lbs)

Instruments

As a Luminex® partner, Merck Millipore is a distributor of Luminex® instruments, accessories and software. We were there at the beginning to help validate Luminex® as a standard of multiplexing, and our ongoing dedication and experience in this technology enable you to gain more information faster, without compromising reliability.

MAGPIX® System

(Cat. No. 40-072 - MAGPIX® System with xPonent 4.2 Software)
(Cat No. 80-073 - **New!** MAGPIX® System with Laptop Option)
(Cat. No. 40-086 - MILLIPLEX® Analyst 5.1 Software - 1 seat license)

Combined with our MILLIPLEX® map magnetic bead-based multi-analyte panels, analysis software and technical support, the Luminex® MAGPIX® system provides a complete solution for rapid, accurate biomarker quantitation in a variety of sample matrices. This affordable system can perform up to 50 tests simultaneously in a single reaction volume, greatly reducing sample input, reagents and labor while improving productivity. The MAGPIX® system features an innovative design based on CCD imaging technology that enables for a more compact, robust system. In addition, it is easy to operate and maintain with streamlined start-up and shutdown protocols and minimal maintenance requirements.

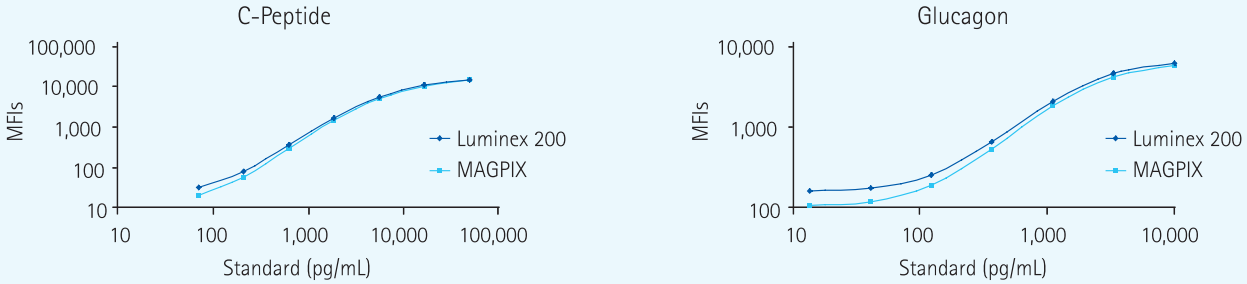
Advantages:

- Powerful - More than 110 (and growing) MILLIPLEX®map magnetic bead-based assay kits-the largest offering of customizable magnetic bead immunoassay panels for the MAGPIX® instrument
- Easy-of-use, magnetic bead-based technology using CCD imaging
- Multiplex - Simultaneously measure up to 50 analytes in as little as 25 µL of sample
- Small footprint - saves space on your lab bench, requiring only 91.44 cm of linear space
- Portable - easily moves from bench to bench, or lab to lab, self installed
- Lower-cost platform- entry level platform with proven xMAP® technology
- More than 25 MILLIPLEX mag Panels - the largest offering of magnetic bead immunoassay panels for metabolic disease, inflammation, toxicity, neuroscience, and cell signaling research

MAGPIX vs. LUMINEX 200

Luminex instrument comparison data

Standard curve for two representative analytes within the MILLIPLEX MAG(magnetic) Human Metabolic Panel



Luminex®200™ System

(Cat No. 40-012- Luminex 200™ System with xPonent 3.1)
(Cat No. 40-086 - MILLIPLEX® Analyst 5.1 Software - 1 seat license)

The Luminex® 200™ System, is a compact lab analysis system that integrates lasers, optics, fluidics, a controller, advanced digital signal processing, an XY Platform, the newest version analysis software, and a PC with monitor. Specifically designed to enhance the use of xMAP® technology, this system enables simultaneous assays of up to 100 analytes per well of a microtiter plate. Key features include: flexible data analysis package, user friendly functions, flexible assay design features, bar code reader for enhanced efficiency and multiple formats for generating data reports.

Luminex 200™ Advantages:

- Flexible - Run both magnetic bead and non-magnetic bead immunoassays.
- Multiplex - Up to 100 analytes per well of a 96 well microtiter plate in as little as 25 µL of sample.
- Ease of use - User-friendly programming functions.
- Scalable - Bar code reader included.

Luminex® FLEXMAP 3D® System

(Cat. No. 40-014 - FLEXMAP 3D® System with xPonent 4.2)
(Cat. No. 40-086 - MILLIPLEX® Analyst 5.1 Software - 1 seat license)

The FLEXMAP 3D®system combines differentially dyed fluorescent microsphere sets with an innovative instrument design that can accommodate measuring the concentrations of up to 500 unique analytes within a single sample rapidly and precisely. Features - such as automated probe height adjustment, simplified routine maintenance operations and an intuitive software interface - make FLEXMAP 3D® a flexible and easy-to-use high-throughput multiplexing platform capable of quantifying protein and nucleic acid biomarkers.

▶ FLEXMAP 3D® System Advantages:

- Automation/LIS compatibility - New xPONENT 4.2 Software controls the FLEXMAP 3D®system and offers automated maintenance routines as well as interfacing options for Laboratory Information Systems (LIS) and other automation platforms.
- Ultra fast - Run 48,000 analytes in less than one hour. Dual sample fluidics paths and increased syringe injection speed facilitates a faster sample injection rate.
- 96- and 384-well capability - Greater sample volume flexibility and increased throughput.
- Multi-functional - Perfect for running both protein and nucleic acid applications on the same instrument.
- Extended Dynamic Range - Linear response of up to at least 400,000 MFI, range is limited by biology, not by the instrument.
- Highest multiplexing - Each microsphere is impregnated with different amounts of three dyes. Monitoring the relative intensities of the three signals enables the system to discriminate up to 500 different microsphere sets.

Washing Solutions for Magnetic and Nonmagnetic Bead Assays

To complete our multiplex assay solution of MILLIPLEX®map kits, instruments, software and service, Merck Millipore offers BioTek® microplate washers, handheld magnetic separation block and MultiScreen® HTS vacuum manifold.

▶ New! 405 LS and 405 TS Microplate Washers for Biomagnetic and Nonmagnetic Beads

(Cat No. 40-094 - BioTek® 405 LS Magnetic 96-well Washer)
(Cat No. 40-095 - BioTek® 405 LS Magnetic/Vacuum Filtration 96-well Washer)
(Cat No. 40-096 - BioTek® 405 TS Magnetic 96-well Washer Complete with Touch Screen and Ultrasonic Cleaning)
(Cat No. 40-097 - BioTek® 405 TS Magnetic/Vacuum Filtration 96-well Washer Complete with Touch Screen and Ultrasonic Cleaning)

In partnership with BioTek®, we now offer the latest advancements in multiplex washing: a fully automated system designed to quickly wash an entire plate through biomagnetic separation, washing and vacuum filtration. Both systems offer magnetic and vacuum filtration options- with the 405 TS model now offering an easy to use and glove usable touch screen. These newest Biotek®Washers come pre-loaded with Merck Millipore-validated wash protocols.

BioTek®plate washer model 405 LS (left) and models 40-094 and 40-095 (right).

BioTek® Washer Advantages:

- Fast and hands-free full plate washing.
- MILLIPLEX® map and Luminex® xMAP®-approved.
- High-energy neodymium iron boron magnets for rapid separation of multiplex beads with superior retention.
- 405 TS models have state-of-the-art, high resolution LED backlit touch screen user interface for intuitive and flexible onboard instrument.
- 405 TS models come with the built in Ultrasonic Advantage™ enabling for easy cleaning even with the toughest of sample types.

▶ BioTek® Magnetic 96-well Strip Washer — Hands-Free Washing With a Small Footprint

(Cat No. 40-062 – BioTek® ELx50 Magnetic 96-well Strip Washer)

Better together: ELx50 Washer with MILLIPLEX® map Magnetic Bead Assays

- Reduced hands-on time for multiplex assays.
- Optimized magnet for strip wells and flat-bottom magnetic bead assay plates.
- Self-contained, programmable washer enables precise fluidic delivery-ensuring complete control while washing multiplex assay plates.

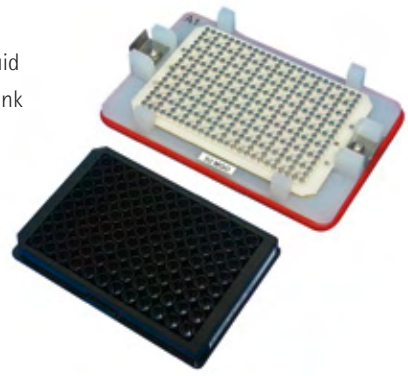
BioTek® Magnetic 96-well strip washer.

▶ Handheld Magnetic Separator Block for 96-Well Flat Bottom or Conical Well Plates

(Cat No. 40-285 – Handheld Magnetic Separator Block for 96-well Flat Bottom or Conical Well Plates)

Merck Millipore offers a low-cost alternative to automated washing of MILLIPLEX® map magnetic immunoassays without loss in assay performance. The handheld magnetic separator allows the liquid contents of the 96-well plate to be removed by simply decanting or "flicking" the contents into a sink and blotting off the remainder on a paper towel. Magnetic beads are securely held to the sides by 9 magnets surrounding each well.

- Top magnetic frame is white polycarbonate, with a corrosion-resistant steel plate underneath, all mounted to a polypropylene base.
- Adjustable clip system holds a wide variety of microplates to the separator block.
- O-Ring on base plate facilitates gripping for all sizes of hands.



▶ MultiScreen® HTS Vacuum Manifold

(Cat No. MSVMHTS00 – MultiScreen® HTS Vacuum Manifold)

Developed for Maximum Versatility

- Configurations for deep well or standard receiver plates
- ANSI/SBS compliant footprint allows for easy robotic deck integration
- Solvent-resistant

The MultiScreenHTS vacuum manifold is ideal for multiwall filter plate applications (e.g. polystyrene beads washing for xMAP® assays) in both manual and automated laboratory environments. The manifold supports a wide variety of MultiScreen platforms, including 96-well and 384-well filter plates for bioassays, and deep well Solvinert™ filter plates for sample preparation. Built with flexibility in mind, it can be easily configured to support applications that require flow to waste as well as analyte collection. As part of the standard equipment, you receive a vacuum pressure indicator allowing you to set and reliably measure vacuum pressure. Controls include an external on/off valve and a vacuum level adjustment valve for optimizing filtration performance. You also have the choice of vacuum source options, including the use of a Millipore vacuum pump, available separately, or house vacuum.

Required Accessories

Chemical Duty Pump 220 volts, 50 Hz - Cat. No. WP61 220 50
Vacuum Flask, 1 L - Cat. No. XX10 047 05
No. 8 stopper, 9.5 mm (3/8 in.) hole, silicone - Cat. No. XX20 047 18

MILLIPLEX® Analyst 5.1 and Luminex® xPONENT® Software

(Cat No. 40-086 - MILLIPLEX® Analyst 5.1 Software – 1 seat license)
(Cat No. 40-087 - MILLIPLEX® Analyst 5.1 Software – 5 seat license)
(Cat No. 40-088 - MILLIPLEX® Analyst 5.1 + Data Base Software – 1 seat license)
(Cat No. 40-089 - MILLIPLEX® Analyst 5.1 + Data Base Software – 5 seat license)

▶ Next generation multiplex data analysis, designed for ease of use and getting the most information out of your data.

Merck Millipore offers the most powerful combination software package, including best-in-class multiplex data analysis with our MILLIPLEX® Analyst 5.1 software coupled with data acquisition using the Luminex xPONENT® software. MILLIPLEX® Analyst 5.1 software enables you to manage, track and analyze your multiplex assays rapidly and efficiently, giving you more time to focus on advancing your research.
Data acquisition and analysis integrates seamlessly with all Luminex® instruments, including FLEXMAP 3D®, Luminex 200™ and MAGPIX® systems. MILLIPLEX® Analyst 5.1 software is available in one- and five-seat licenses, enabling complete flexibility for small, medium and large laboratories.

▶ The most advanced curve fitting algorithm, based on self-learning improvements using real-life data sets.

Analyzing data from multiplexed biomarker assays can be difficult when working with diverse sample and analyte types. This diversity can lead to a wide range of possible analyte levels and assay signal intensity with respect to those analyte levels, both of which are not always easy to predict or determine accurately. MILLIPLEX® Analyst 5.1 software was designed to generate the most meaningful quantitative analyte data with a focus on data derived from the low and high ends of standard curves. Data in these regions can be important and are commonly missed by existing multiplex data analysis packages.
In developing the new curve fitting algorithms for MILLIPLEX® Analyst® 5.1, simulations were run on 600+ data sets using actual experimental standard curves to determine the curve fit that would give the lowest CVs at the low and high ends of the curves and that works well even with standard curves of low quality.

▶ Better curve fit, higher recoveries and more reliable data at the low and high ends of your standard curves: See what you've been missing!

Most multiplex data analysis tools can calculate concentrations in the middle of the curve, but may struggle with the data from the plateaus. Improved curve-fitting algorithms and weighting methods in MILLIPLEX® Analyst 5.1 software resulted in the software's ability to generate more meaningful data points at high and low concentration extremes for many biologically important analytes, typically outperforming other commonly used multiplex data analysis software packages.

Analyte: IL-28B, Kit: Mouse TH17
Units: pg/mL

	MILLIPLEX® Analyst 5.1*	StatLIA®	Bio-Plex®
Standard1	134.2	127.0	127.3
Standard2	470.5	503.0	502.8
Standard3	2099.0	2073.0	2072.8
Standard4	8047.0	7887.0	7887.9
Standard5	32635.0	34418.0	34409.9
Standard6	129603.0	121675.0	121695.5
Unknown1	**113.5**	**99.0**	**OOR <**
Unknown2	2744.0	2684.0	2684.0
Unknown3	2566.0	2514.0	2514.5
Unknown4	10125.0	10018.0	10018.6
Unknown5	**107.0**	**90.0**	**OOR <**
Unknown6	**126.3**	**117.0**	**OOR <**
Unknown7	**93.6**	**70.0**	**OOR <**
Unknown8	**93.6**	**70.0**	**OOR <**
Unknown9	**107.0**	**90.0**	**OOR <**
Unknown10	**126.3**	**117.0**	**OOR <**
Unknown11	**105.3**	**87.0**	**OOR <**
Unknown12	205.4	215.0	215.3
Unknown13	160.2	161.0	160.6
Unknown14	**121.6**	**110.0**	**OOR <**
Unknown15	**118.4**	**106.0**	**OOR <**
Unknown16	**121.6**	**110.0**	**OOR <**
Unknown17	**123.2**	**113.0**	**OOR <**
Unknown18	142.0	138.0	137.4
Unknown19	**98.7**	**78.0**	**OOR <**
Unknown20	**102.0**	**83.0**	**OOR <**
Unknown21	**107.0**	**90.0**	**OOR <**
Unknown22	**118.4**	**106.0**	**OOR <**
Unknown23	**98.7**	**78.0**	**OOR <**
Unknown24	**120.0**	**108.0**	**OOR <**

Blue: Extrapolated value
OOR<: Out of Range Below
*Best Fitting, 5P lin weighted

Table 1.
Significantly more IL-28 concentrations could be calculated at the low end of the curve in the Mouse TH17 Magnetic Bead Panel by MILLIPLEX® Analyst 5.1 software compared to StatLIA® (extrapolated values) and Bio-Plex® software packages. Data from application note, "Improved analysis of multiplexed biomarker quantitation data with MILLIPLEX® Analyst 5.1 software," Lit No. AN5664ENEU.

Enhanced features of MILLIPLEXR Analyst 5.1 software include:

- MILLIPLEX® Analyst 5.1 software offers simple integration with Luminex xPONENT® system.
- Seamless loading of data via a Quick Start Wizard.
- Automatic importing of data from Luminex® instruments using our novel Watchdog feature.
- Our superior algorithms yield better data from the low- and high-end of the standard curve.
- Comprehensive detailed reports and enhanced visualization features.

New features and improvements of MILLIPLEXR Analyst 5.1 software:

- Complete with CD and Dongle USB/Thumb drive for quick and easy installation.
- Auto-detect feature for standards, quality controls, and samples.
- Easily analyze data from multiple sources including Luminex® 1.7 files formats, Microsoft Excel®, Bio-Plex®, and other Luminex® data analysis related software programs.
- The best curve fit possible using our best-in-class algorithms.
- Qualitative analysis allows for relative comparisons between samples and analytes.
- Curve Potency feature (comparing two or more standard curves).
- Create custom formats for importing data.
- Export compatible – PDF and Microsoft Excel® formats.
- Reports values for CHI, Recovery, and CVs.
- Even very large data sets are calculated in seconds.
- Full support of Microsoft Windows® 7, 32-bit and 64-bit.
- Complete support from our world-class technical service team.

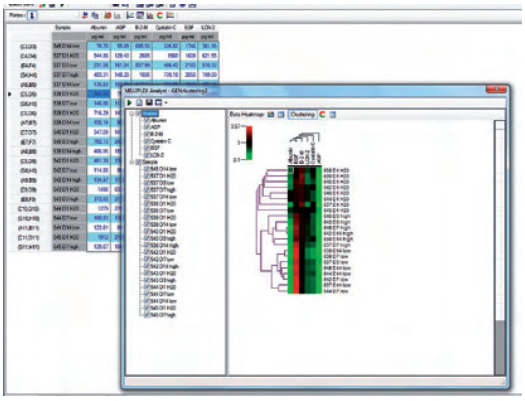


Figure 2.
Hierarchical cluster (based on Pearson correlation coefficients) generated by MILLIPLEX® Analyst 5.1 software.

MILLIPLEX® Analyst 5.1 Detailed Report

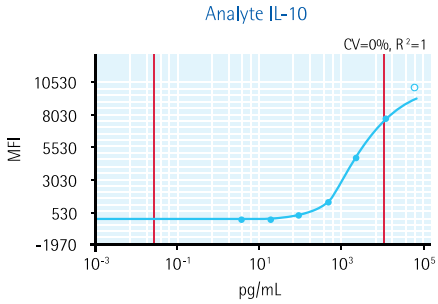
Easily export complete multiplex data for use in presentations and record keeping.

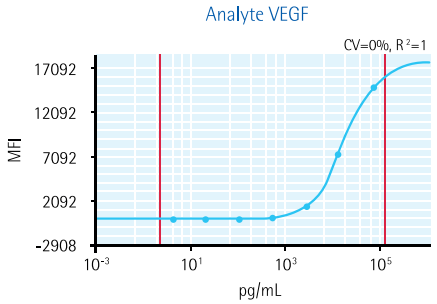
Location	Expected pg/mL(i)	MFI(i)	pg/mL(i)	MFI	pg/mL	CV	Recovery
1C1	0.13	45	0.09	46.5	0.13	4.56%	100.37%
1D1		48	0.17				
1E1	0.64	66	0.68	64.5	0.63	3.29%	99.08%
1F1		63	0.59				
1G1	3.2	164	3.48	153.25	3.17	9.92%	99.12%
1H1		142.5	2.86				
1A2	16	592	16.17	610.75	16.74	4.34%	104.6%
1B2		629.5	17.3				
1C2	80	2456	76.33	2578	80.51	6.67%	100.64%
1D2		2699	84.73				
1E2	400	9938	411.35	9154	367.15	12.11%	91.79%
1F2		8370	325.53				
1G2	2000	23685	2131	23298	2031	2.35%	101.53%
1H2		22912	1936				

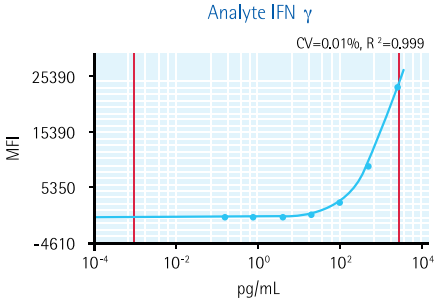
Samples

Location	Sample	MFI(i)	pg/mL(i)	MFI	pg/mL	CV
1A3	QC1	1630	48.75	1599	47.72	2.81%
1B3		1567	46.69			
1C3	QC2	8054	309.44	8581	336.49	8.69%
1D3		9108	364.64			

Figure 3.
Detailed reports generated by MILLIPLEX® Analyst 5.1 software.







5-P.linear.weighted(41.71, 53815.74, 0.01, 3317.51, 0.98)
Chi=3.65%, CV=0.010%, R²=0.999, DC=(0.00085, 2660)

Notes:
CV-The Coefficient of Variation of standard curve replicates at each dilution level.
Chi-The Chi-Square test statistic of the distance between observed concentrations with expected concentrations.

MILLIPLEX® MAP Multiplex Assay Kits

Your Source for Simultaneous Multi-Analyte Detection - The Voice of Luminex® xMAP® Technology

MILLIPLEX® MAP assays offer the broadest selection of multiplex kits and Reagents in a wide variety of research areas, measuring multiple biomarkers using a small sample size. MILLIPLEX® MAP enables the simultaneous detection of multiple soluble or intracellular biomarkers. Using the Luminex MAP® bead-based technology, these flexible and customizable assays are exhaustively tested and qualified for sensitivity, specificity, reproducibility and wide dynamic range.

- Based on Luminex® xMAP® technology and 25 years of protein detection experience.
- The broadest selection of analytes across a wide range of disease states, including metabolic disease, immunology, neurodegenerative disease, toxicity, cancer and more.
- All the components and reagents you need to detect multiple analytes simultaneously in a small sample size (10-50 µL) - all in a single kit, using a single catalogue number.
- Available in magnetic bead format. Magnetic polystyrene beads contain encapsulated magnetite with bead surface chemistries that are comparable to non-magnetic beads. New magnetic bead panels will be introduced into our portfolio each quarter!
- Select a premixed kit or choose analytes within a panel to design a custom kit.
- Quality controls provided to qualify assay performance.
- Analytically validated panels that yield consistent analyte profiles irrespective of plex size.
- Standardized standard curve ranges across analytes and lots to ensure lot-to-lot consistency.
- Panels meet stringent manufacturing criteria to ensure batch-to-batch reproducibility.
- Environmentally friendly packaging: our boxes are 100% recyclable to reduce ecological footprints.
- Consolidated packaging available for larger orders to reduce storage needs.

To learn more, please visit "www.merckmillipore.com/bmia"

Ordering Information

Description	Cat. No.
Accessories	
MAGPIX® Drive Fluid 4PK	MPXDF-4PK
MAGPIX® Calibration Kit (25 doses)	40-049
MAGPIX® Performance Verification Kit (25 doses)	40-050
Luminex® 200 and FLEXMAP 3D® Sheath Fluid (20 L)	SHEATHFLUID
Luminex® 200 Calibration Kit (25 doses)	40-275
Luminex® 200 Performance Verification Kit (25 doses)	40-276
FLEXMAP 3D® Calibration Kit (25 doses)	40-028
FLEXMAP 3D® Performance Verfication Kit (25 doses)	40-029
MILLIPLEX Microtiter Plate for Magnetic beads 2/pk	MAG-PLATE
MultiScreenHTS Microtiter Filter Plate, 2/pk	MX-PLATE
MultiScreenHTS BV Microtiter Filter Plate, 1.2 µm, 10/pk	MSBVN1210
MultiScreenHTS BV Microtiter Filter Plate, 1.2 µm, 50/pk	MSBVN1250

Luminex System 1 Year Warranty Plans				
Warranty Plans	**Visits for emergency repair**	**Emergency repair costs**	**1 (PM*)**	**Cat. No.**
FLEXMAP 3D®, Bronze	None	20% discount off parts only		SVCLUMBRZFM3D
FLEXMAP 3D®, Silver	Unlimited	Travel & Labor only	*	SVCLUMSLVFM3D
FLEXMAP 3D®, Gold	Unlimited	All costs included	*	SVCLUMGLDFM3D
Luminex 200™, Bronze	None	20% discount off parts only		SVCLUMBRZ
Luminex 200™, Silver	Unlimited	Travel & Labor only	*	SVCLUMSLV
Luminex 200™, Gold	Unlimited	All costs included	*	SVCLUMGLD
MAGPIX®, Standard	Unlimited	All costs included		SVCLUMGLDMAGPIX
MAGPIX®, Gold	Unlimited	All costs included	*	SVCMAGPIXGOLDPM

*1 (PM) = includes 1 preventive maintenance

Supported Countries	Gold (LX200/FM3D)	Silver (LX200/FM3D)	Bronze (LX200/FM3D)	Gold (Magpix)	Standard (Magpix)
Czech Republic	•	•	•	•	•
Estonia	•			•	•
Hungary	•			•	•
Latvia	•			•	•
Lithuania	•			•	•
Poland	•	•	•	•	•
Slovakia	•			•	•

Spectrometry

Direct Detect™ Protein Quantitation System

With Direct Detect™, the first infrared (IR)-based Protein/Peptide quantitation system, just spot 2 µL of your sample, blank, and read! No sample prep, no tedious standard curves, and no messy cuvettes or liquid waste. The Direct Detect® system provides more accurate results without the pitfalls of colorimetric assays. By measuring amide bonds in protein chains, the system accurately determines an intrinsic component of every protein without relying on amino acid composition, dye binding properties or reduction-oxidation (redox) potential. You can accurately analyze components of complex mixtures, because proteins, lipids and carbohydrates have separable IR spectra—making lysates and membrane preps easier than ever!

- IR spectrometry measures amide bond absorbance for higher reproducibility across all proteins and peptides
- Improves speed and accuracy over traditional colorimetric assays
- Works in the presence of detergents and reducing agents
- Preserves precious samples—requires only 2 µL per analysis
- Can detect other biomolecules, such as lipids, for analyzing complex mixtures

FTIR Spectrometry and Protein Quantitation

By measuring amide bonds in protein chains, the system accurately determines an intrinsic component of every protein without relying on amino acid composition, dye binding or redox potential. Accurately analyze components of complex mixtures: proteins, lipids, carbohydrates, and nucleic acids have separable IR spectra.

Advantages

- Independent of amino acid composition
- No extinction coefficient required, unlike UV-Vis/A280 methods
- Improves speed and accuracy over traditional colorimetric assays
- Works in the presence of detergents and reducing agents
- Compatible with lysates and membrane preps

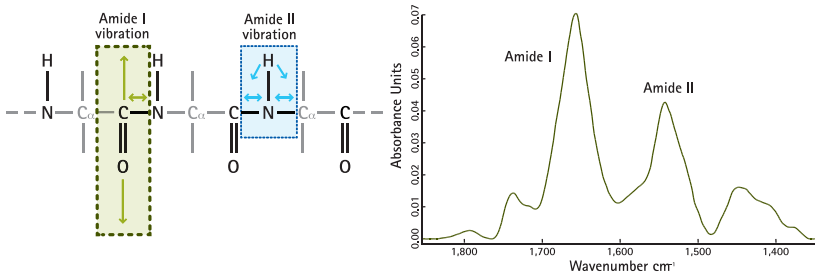


Figure 1.

Vibrations responsible for the Amide I and Amide II bands in the infrared spectra of proteins.

How the Direct Detect™ Spectrometer Gives You Better Quantitation

- No need for colorimetric assays, giving you speed, accuracy and reproducibility
- Works even in the presence of detergents and reducing agents
- IR spectrometry measures amide bond absorbance for better accuracy across all proteins and peptides
- Requires minimal sample volume (2 µL), preserving precious samples

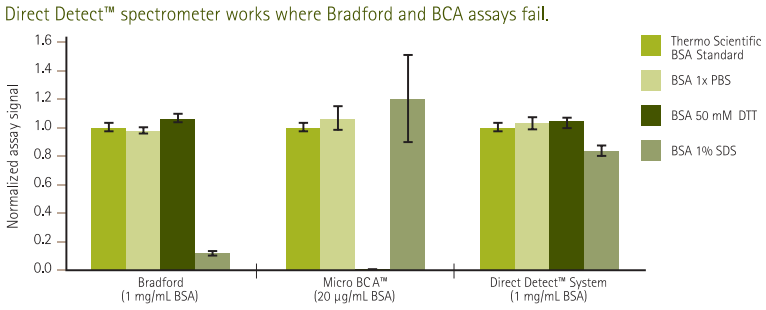


Figure 2.

The Direct Detect™ spectrometer provides accurate and precise results, even in presence of detergent (SDS) and reducing agent (DTT). Using the Direct Detect™ IR-based quantitation system, calculated concentrations for all the BSA samples matched the prediluted standard (Thermo Scientific BSA Standard).
In comparison, the Coomassie Plus (Bradford) Assay provided calculated concentrations that differed greatly in the presence of 1% SDS, and the Micro BCA™ assay could not provide data in the presence of 50 mM DTT.

▸ **Direct Detect spectrometer surpasses current approaches to quantitation**

	UV/Vis and Colorimetric	Direct Detect™ Spectrometer	Advantages of Direct Detect™ Spectrometer
Absorbance mode	UV/Vis	Infrared	• Intrinsic measurement of amide bonds • No extinction coefficient • No need for colorimetric/fluorimetric assays
Biomolecules detected	Protein	Protein/Peptide	• Provides confidence of sample purity • Peptide analysis (not possible with UV) • More information from less sample
Assay compatibility	Colorimetric (Bradford/BCA)	No assay required	• Saves time • More accurate/reproducible
Small sample volume	Some instruments	Yes	• Not possible to get accurate results in colorimetric assay with small volume/ amount

The Key to Simple Analysis: Membrane Technology

The Direct Detect™ system exploits a new membrane technology that makes aqueous biological samples compatible with direct infrared analysis. The membrane card employs a hydrophilic polytetrafluoroethylene (PTFE) membrane that is transparent in most of infrared spectral region, so you can spot tiny volumes of your biomolecule solutions directly onto the membrane. No sample prep, no need to run a standard curve every time, no cuvettes, and no liquid waste. Spotted samples are stable in ambient conditions, so you'll get reproducible data, whether you read the card in the next hour or next month.

Direct Detect™
Assay-free Cards

Specifications

- **Dynamic range: 0.2 ~ 5 mg/mL**
- **Peptide / Protein size: Minimum 3 residues**
- **Hydrophilic PTFE membrane: Broad chemical compatibility transparent in IR spectrum**
- **Sample Volume (per spot): 2 uL**

Performances

• Matches Amino Acid Analysis	• Samples Containing Detergents
• Buffer Compatibility	• Precise Quantitation

Dynamic Concentration Range and Accuracy

The Direct Detect™ system is recommended for use with protein samples within the range 0.2 mg/mL - 5 mg/mL. Although the instrument can measure protein concentrations from 0.1 mg/mL to around 30 mg/mL, the most accurate results are achieved within the recommended concentration range.

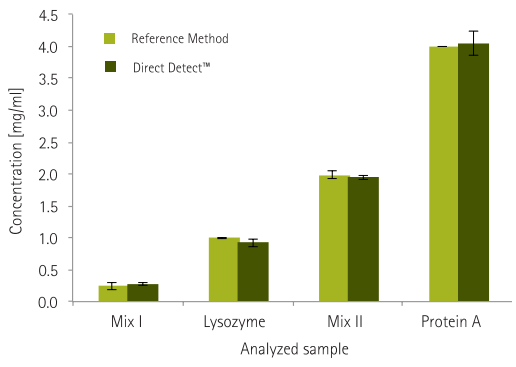


Figure 3.

The Direct Detect™ system provides protein quantitation in agreement with amino acid analysis (AAA). Direct Detect™ concentration determination performed within system dynamic range and compared to reference method (AAA). Error bars represent standard deviations of triplicate sample spots (Direct Detect™) or readings (AAA).

■ Dynamic Concentration Range and Accuracy

Direct Detect™ provides accurate and precise results, even in presence of detergent (SDS) and reducing agent (DTT). Using the Direct Detect™ IR-based quantitation system, calculated concentrations for all the BSA samples matched the prediluted standard (Thermo BSA Standard). In comparison, the Coomassie Plus (Bradford) assay provided calculated concentrations that differed greatly in the presence of 1% SDS, and the MicroBCA assay could not provide data in the presence of 50 mM DTT..

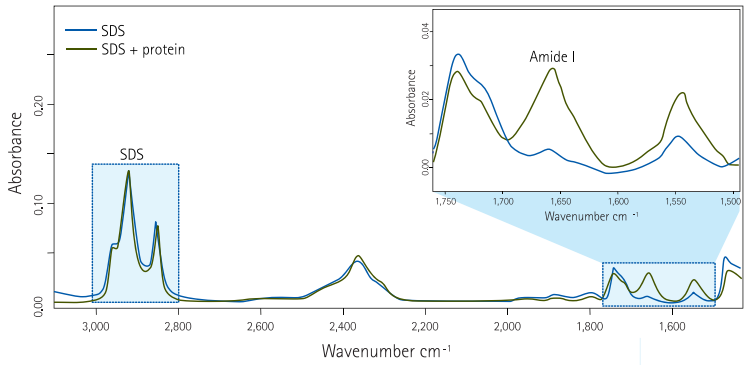


Figure 4.
SDS does not interfere with Direct Detect™ quantitation.
The IR spectrum of SDS alone (blue) does not contribute appreciably to the intensity of the Amide I peak in the IR spectrum of SDS + protein (green).

▶ Requires less sample and more compatible with buffer components compard to colorimetric assays

Quantitation Approach	Assay Range	Sample Volume (tube/plate)	Amount Protein Required to Measure 1 mg/mL	Compatible	Incompatible
Direct Detect™ System	200-5,000 µg/mL	2 µL	6 µg	Chelators Detergents Reducing agents SDS	(High) Urea
Bradford Assay	100-1,500 µg/mL	35 µL/7 µL	21 µg	Chelators Most reducing agents	Detergents
BCA Assay	20-2,000 µg/mL	50 µL/25 µL	30 µg	Detergents	Chelators Reducing agents
Micro BCA™ Assay	2-40 µg/mL	500 µL/150 µL	450 µg	Detergents	Chelators Reducing agents
Lowry Assay	10-1,500 µg/mL	200 µL/40 µL	120 µg	SDS	Chelators Detergents Reducing agents

■ Reproducibility and Precision

In contrast to other currently available methods for estimating protein concentration, Direct Detect™ enables the user to perform multiple measurements without additional sample requirement. The assay free sample card permits multiple analyses of the same sample that can be separated by hours or days.

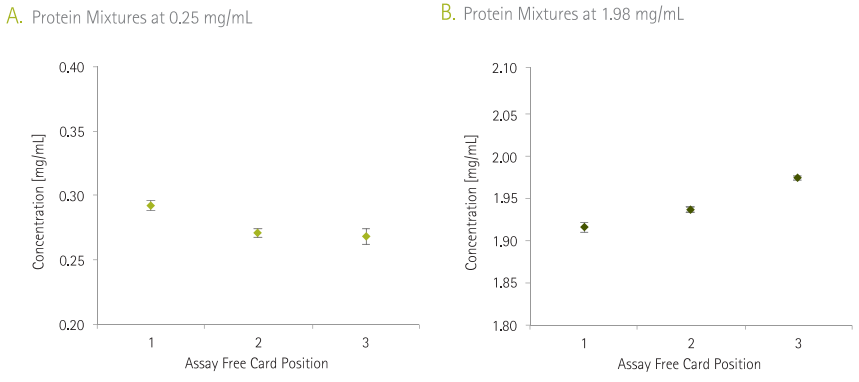
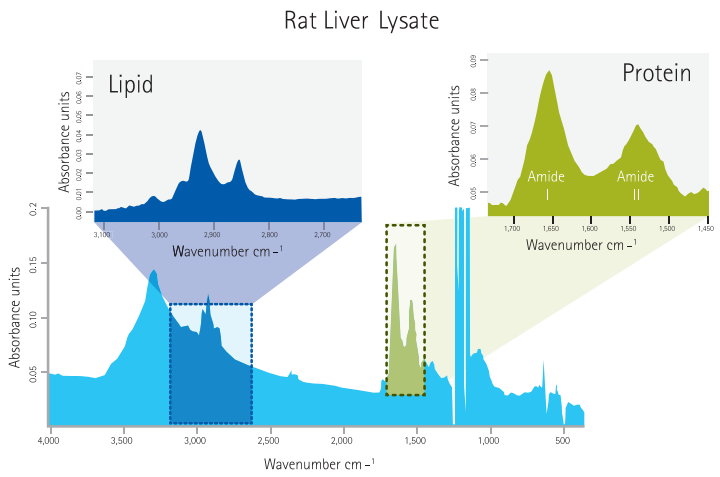


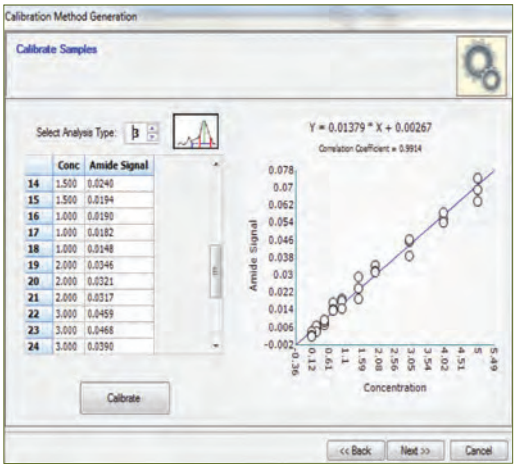
Figure 5.
The Direct Detect™ system delivers precise concentration analysis of protein mixtures at 0.25 mg/mL (A) and 1.98 mg/mL (B). Each data point is represented by four independent measurements. Error bars represent standard deviation.



Complex Samples?

With Direct Detect™ IR-based technology, you can accurately analyze components of complex biological samples, including proteins, lipids, carbohydrates, and nucleic acids separable via IR spectra. Lysates and membrane preps are easier than ever!

Protein quantitation in presence of lipids within a complex cell lysate is possible because the most intense regions of lipid absorbance are spectrally distinct from the protein's Amide I and Amide II signals.



Standard Curve Generation.

The Direct Detect™ Protein quantitation system was calibrated using NIST-certified BSA. A series of ten dilutions (in triplicates) spanning the range from 0.125 mg/mL to 5 mg/mL was used to prepare a robust calibration curve.

The intensity of the amide signal delivered at each concentration point was fitted to a regression line represented by linear equation y = 0.01379x + 0.00267. The equation was further used by Direct Detect™ software to determine protein concentration in the analyzed samples.

Ordering Information

Description	Qty	Cat. No.
Direct Detect™ Spectrometer and Starter Kit	**1**	**DDHW00010-WW**
Includes:	**1**	
Direct Detect™ Spectrometer	1	
Universal Power Adapter	1	
Dell Latitude™ 2120 Netbook and power adapter	1	
Direct Detect™ Software	1	
Netbook Stand	1	
Spotting Tray	1	
Ethernet Cable	1	
Direct Detect™ Assay-free Cards (50/pk)	1	
Direct Detect™ Assay-free Cards (50/pk)	1	DDAC00010-GR
Direct Detect™ Assay-free Cards (50/pk)	4	DDAC00010-4P
Direct Detect™ Assay-free Cards (50/pk)	8	DDAC00010-8P
Additional Warranty		
1 year additional warranty, at time of purchase		DDWAR0010-10
1 year additional warranty, after purchase		DDWAR0010-11
2 year additional warranty, at time of purchase		DDWAR0010-20
2 year additional warranty, after purchase		DDWAR0010-21
Additional year of warranty, at purchase		DDWAR0010-30
Additional year of warranty, after purchase		DDWAR0010-31

To learn more, please visit "www.merckmillipore.com/directdetect"

Goodbye, Bradford Assays!

Drive your research forward with IR-based quantitation.

Western Blotting & IHC

SNAP i.d.® 2.0 system for Western Blotting and IHC

There's so much room for experimental variability in traditional immunodetection workflows. For your peace of mind - and ours - we designed the SNAP i.d.® 2.0 system to streamline your Western blot and immunohistochemistry experiments.

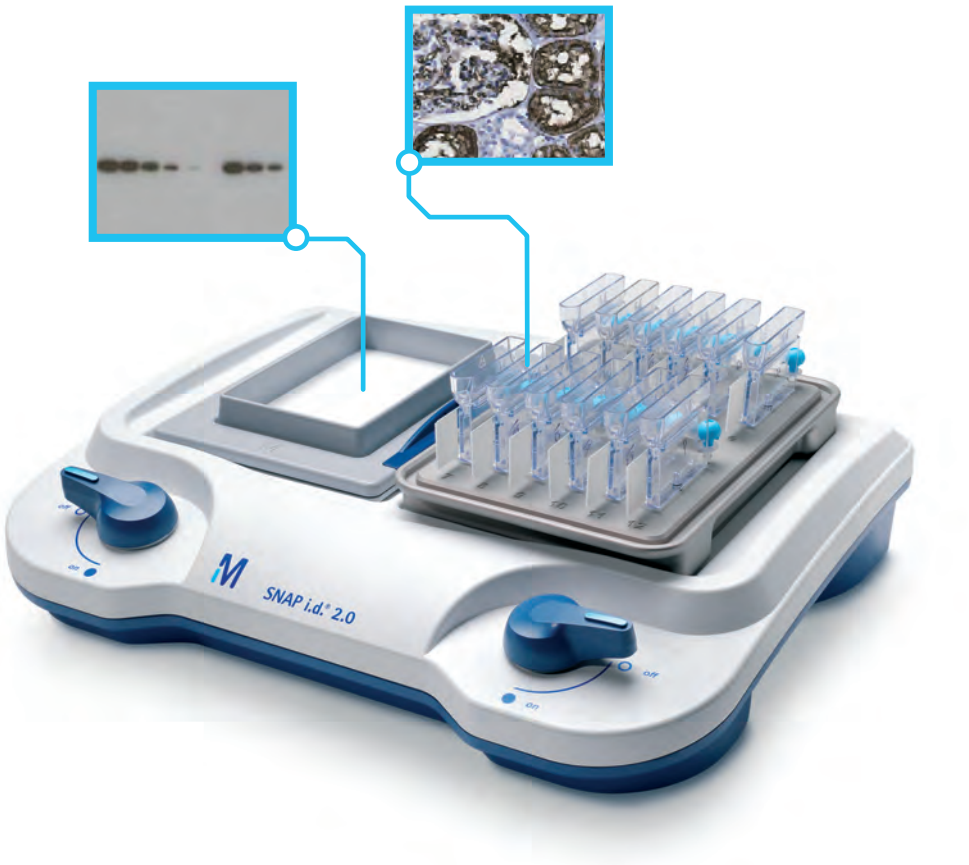
The concept is simple: a vacuum-driven flow of blocking, antibody, and washing solutions reduces slide and membrane handling. That means a lot less shaking, dipping, pouring and waiting. And now you can process multiple blots and slides in parallel, so it's easy to apply consistent conditions across experiments.

SNAP i.d.® 2.0 for Western Blotting:

- Superior - Increased antibody-antigen binding, enhanced washes, and antibody recollection
- Flexible - Three gel sizes: multiblot (4.5 x 8.5), mini (7.5 x 8.4 cm) and midi (8.7 x 13.5 cm)
- Faster results - 30 minute immunodetection

SNAP i.d.® 2.0 for IHC:

- Eliminates the need for pap pens
- Antibodies can be collected and reused
- Slide handling time is significantly decreased
- Less time spent on wash steps
- Parallel processing of multiple slides



Parts and Functions of the SNAP i.d.® 2.0 System

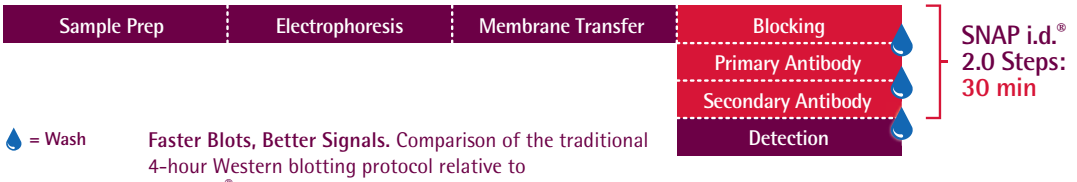
Your SNAP i.d.®2.0 system includes everything you need to get started, including the detection base, blot holders (2x), two independent blot holder frames (come with lids), antibody collection tray (2x), vacuum tubing, rolling pad, wetting trays (2x) and blot roller. In addition, we provide you with an integrated vacuum regulator to ensure consistent vacuum pressure without the need for an external regulator.

Other useful accessory hardware include: Chemical duty pump (system requires vacuum source that can deliver a sustained minimum pressure of 410 millibar (12 in. Hg) and 34 L/min)) a one-liter Vacuum filtering flask, Silicone No. 8 perforated stoppers (5 to a pack) and stainless steel forceps.

Components of the SNAP i.d.® 2.0 System-Mini, Midi or MultiBlot:

	Part	Function
A	Base Unit (1)	Accepts frames and facilitates immunodetection
B	Tubing Assembly Kit (1)	Connects system to vacuum source
C	Blot roller (1)	Eliminates air bubbles between the blot and blot holder
D	Rolling pad (1)	Provides smooth surface for assembling blot
E	Wetting tray (2)	Used for wetting out blot holder and blot
F	Antibody Collection Tray (2)	Collects antibody for recycling
G	Snap i.d.® 2.0 Mini Blot Holding Frame and Lid	Accepts Mini blot holders for blot incubation
H	Snap i.d.® 2.0 Midi Blot Holding Frame and Lid	Accepts Midi blot holders for blot incubation
I	Snap i.d.® 2.0 MultiBlot Holding Frame and Lid	Accepts MultiBlot holders for blot incubation
J	Snap i.d.®2.0 Mini Blot Holder	Accepts up to 7.5 x 8.4 cm blots
K	Snap i.d.®2.0 Midi Blot Holder	Accepts up to 8.5 x 13.5 cm blots
L	Snap i.d.® 2.0 MultiBlot Holder	Accepts up to 4.5 x 8.5 cm blots
SNAP i.d. Accessory Hardware		
M	Chemical Duty Pump, 220 V/50 Hz	Vacuum Source
N	Vacuum filtering flask, 1 L	Waste collection
O	No. 8 perforated stopper, silicone	
P	Tubing, 3/16 in. ID x 140 cm, silicone	
R	Filter forceps, blunt end, stainless steel	Safe handling of membranes
S	Millex-FA50 filter	Protection the vacuum source from contamination

SNAP i.d.® 2.0 system in the Western blotting workflow



Faster Blots, Better Signals. Comparison of the traditional 4-hour Western blotting protocol relative to SNAP i.d.® 2.0 system's 30 minute protocol.

The SNAP i.d.® 2.0 Protein Detection system is the second generation of the new gold standard for the immunodetection phase of Western blotting. This unique system dramatically enhances Western blot throughput by accelerating the entire immunodetection process (i.e., blocking, washing, and primary and secondary antibody incubations) from >4 hours to only 30 minutes. As in many other techniques, each antibody needs to be optimized for the SNAP i.d.® 2.0 system to ensure optimal performance.

Vacuum Transport

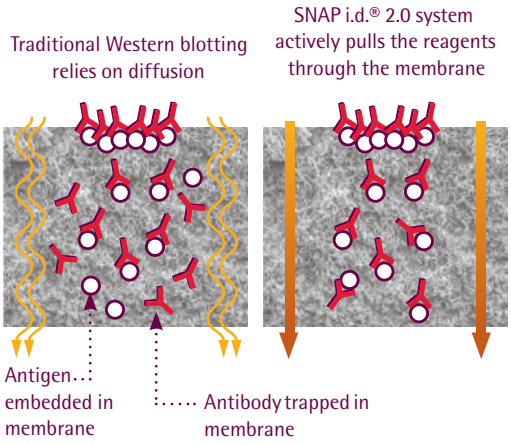
SNAP i.d.® 2.0 System: Decrease immunodetection time by 80% compared to traditional Western blotting

This novel method allows you to optimize your Western blotting conditions in record time for maximum results. The SNAP i.d.® 2.0 system is compatible with fluorescent, chemiluminescent or chromogenic detection. Moreover, the sequence of steps required to process a Western blot with the SNAP i.d.® 2.0 system is identical to that used in traditional immunodetection. Because it uses a vacuum to actively drive reagents through the membrane, blocking and washing steps are faster and more thorough.

Advantages of the SNAP i.d.® 2.0 System's Vacuum Transport Feature

Traditional immunodetection relies on the slow diffusion of reagents into and out of the blot, leading to long incubation times and possible high background. The SNAP i.d.® 2.0 system actively pulls the antibodies through the membrane for maximum interaction with the antigens without a residual high background. The system increases local antibody concentrations at binding sites by using vacuum filtration as well as decreased antibody volumes, driving the antibody-antigen binding reaction forward and shortening incubation times. Vacuum pulls any residual, unbound antibody out of the membrane, lowering background signal.

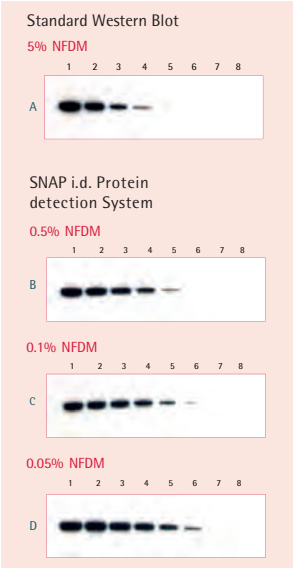
- Draws reagents through blotting membrane
- Minimizes over-blocking
- Membranes are thoroughly flushed instead of just rinsed
- Reduced incubation times



Low concentrations of blocking reagent with SNAP i.d. improve quality & signal

Use low concentrations of blocking reagents with the SNAP i.d. system to improve quality.

Non-Fat Dry Milk (NFDM) is an efficient blocking solution commonly used in Western blotting; however, its high blocking capacity may compromise the protein signal. To demonstrate this, a two-fold dilution series of rat liver lysate (12 µg in lane 1 to 0.09 µg in lane 8) was resolved with SDS-PAGE prior to blotting and immunodetection. (The primary antibody was mouse anti-Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH); the secondary antibody was HRP-conjugated goat anti-mouse). Blot A used a standard immunodetection protocol (block for 1 hour in 5% NFDM, incubate in primary (1:40,000) or secondary antibody (1:50,000) for one hour, wash three times following incubations). Blot B, C and D were assembled in SNAP i.d blot holders and blocked for 20 seconds with either 0.5, 0.1 or 0.05% NFDM respectively. The blots were incubated for 10 minutes with anti-GAPDH (1:13,000), washed immediately and incubated for 10 minutes with HRP goat anti-mouse (1:10,000). Results show an increase in sensitivity with a decrease in milk concentration.



Antibody Optimization

Typically, researchers lack the time to optimize their blotting protocols. By shortening the time required for blocking, washing and antibody incubations to 30 minutes, the SNAP i.d.® 2.0 system allows you to optimize your immunodetection conditions for the highest quality results. Three blot holder sizes accommodates gels of different size and two maximally four blot holders can be run in parallel. Thus, you can quickly optimize conditions and greatly increase your protein detection throughput.

To save even more of your time, we have created reference guide of antibodies with optimized working concentrations for the SNAP i.d.® 2.0 system. This guide will be updated periodically with new working concentrations for a growing list of antibodies. For a complete listing, visit the SNAP i.d.® 2.0 System Antibody Optimization Reference Guide at: www.merckmillipore.com/snapab

Ordering Information

Description	Qty	Cat. No.
SNAP i.d.® 2.0 Western Blotting Systems		
SNAP i.d.® 2.0 System-Mini (7.5 x 8.4 cm)		SNAP2MINI
SNAP i.d.® 2.0 System-Midi (8.5 x 13.5 cm)		SNAP2MIDI
SNAP i.d.® 2.0 System-MultiBlot (4.5 x 8.4 cm)		SNAP2MB3
SNAP i.d.® 2.0 System-Mini and Midi		SNAP2MM
SNAP i.d.® 2.0 System-Mini and MultiBlot		SNAP2MB1
SNAP i.d.® 2.0 System-Mini and MultiBlot		SNAP2MB2
SNAP i.d.® 2.0 Consumables		
SNAP i.d.® 2.0 Mini Blot Holders (7.5 x 8.4 cm)	100/pk	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders (8.5 x 13.5 cm)	100/pk	SNAP2BHMD0100
SNAP i.d.® 2.0 MultiBlot Holders (4.5 x 8.4 cm)	50/pk	SNAP2BHMB050
SNAP i.d.® 2.0 MultiBlot Holders (4.5 x 8.4 cm)	200/pk	SNAP2BHMB-K
SNAP i.d.® 2.0 Accessories		
SNAP i.d.® 2.0 Mini Blot Holding Frames (single pack)	1/pk	SNAP2FRMN01
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack)	1/pk	SNAP2FRMD01
SNAP i.d.® 2.0 MultiBlot Holding Frames (single pack)	1/pk	SNAP2FRMB01
SNAP i.d.® 2.0 Mini Blot Holding Frames (double pack)	2/pk	SNAP2FRMN02
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack)	2/pk	SNAP2FRMD02
SNAP i.d.® 2.0 Antibody Collection Tray	20/pk	SNAPABTR
SNAP i.d.® 2.0 Blot Roller	1/pk	SNAP2RL
SNAP i.d. Accessory Hardware		
Chemical Duty Pump, 220 V/50 Hz	1	WP6122050
Vacuum filtering flask, 1 L	1	XX1004705
No. 8 perforated stopper, 9.5 mm hole, silicone	5	XX1004718
Tubing, 3/16 in. ID x 140 cm, silicone	1	XX7100004
Filter forceps, blunt end, stainless steel	3	XX6200006P
Blotting Membranes		
Immobilon® -P PVDF, 0.45 µm, 26.5 x 3.75 cm roll	1 roll	IPVH00010
Immobilon® -P PVDF, 0.45 µm, 7 x 8.4 cm sheet	50/pk	IPVH07850

Description	Qty	Cat. No.
Immobilon®-P PVDF, 0.45 µm, 8.5 x 13.5 cm sheet	10/pk	IPVH08130
Immobilon®-FL PVDF, 0.45 µm, 26.5 x 3.75 cm roll	1 roll	IPFL00010
Immobilon®-FL PVDF, 0.45 µm, 7 x 8.4 cm sheet	10/pk	IPFL07810
Immobilon®-PSQ PVDF, 0.2 µm, 7 x 8.4 cm sheet	50/pk	ISEQ07850
Immobilon®-P PVDF Sandwich, 0.45 µm, 7 x 8.4 cm	20/pk	IPSN07852
Immobilon®-P PVDF Sandwich, 0.45 µm, 8.5 x 13.5 cm	20/pk	IPSN08132
Reagents		
Luminata™ Forte Western HRP Substrate	500 mL	WBLUF0500
Luminata™ Crescendo Western HRP Substrate	500 mL	WBLUR0500
Luminata™ Classico Western HRP Substrate	500 mL	WBLUC0500
Immobilon® Western HRP Substrate	500 mL	WBKLS0500
Visualizer™ Western Blot Detection Kit, mouse	1 kit	64-201
Visualizer™ Western Blot Detection Kit, rabbit	1 kit	64-202
Calbiochem® TMB, insoluble	100 mL	613548
Calbiochem® SignalBoost™ Immunoreaction Enhancer Kit	1 kit	407207
Immunoblot Blocking Reagent	20 g	20-200
ReBlot™ Plus Mild Antibody Stripping Solution, 10x	50 mL	2502
Re-Blot™ Plus Strong Antibody Stripping Solution, 10X	50 mL	2504
bløk®-CH Buffer	500 mL	WBAVDCH01
bløk®-FL Buffer	500 mL	WBAVDFL01
bløk®-PO Buffer	500 mL	WBAVDP001
Novagen® 5% Alkali-soluble Casein	225 mL	70955

To learn more, please visit "www.merckmillipore.com/SNAP"

SNAP i.d.® 2.0 IHC System

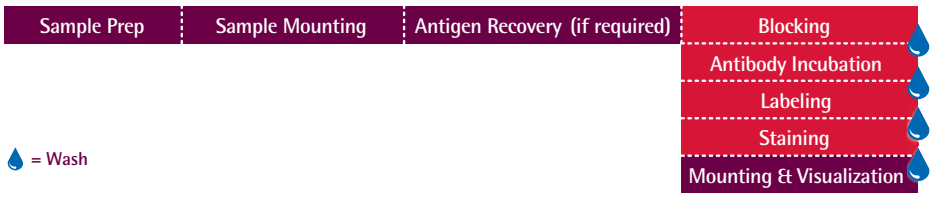
Issues with your tissues? Process all your slides in a SNAP for streamlined IHC

Variability - it's the challenge you face in traditional immunohistochemistry (IHC) experiments. If you're handling slides manually, using pap pens, pipetting, dipping and pouring, you risk slide-to-slide process variability, which may affect your results. The SNAP i.d.® 2.0 Protein Detection System for Immunohistochemistry (IHC) introduces a new capability to the innovative, vacuum-driven SNAP i.d.® 2.0 system.

The IHC slide holders allow you to block, probe, and stain up to 12 tissue slides (designed for standard 25 x 75 x 1 mm glass slides) per side (24 slides if both sides are used). Reduced handling time and multiple-slide processing make this system ideal for when you are optimizing antibody conditions and protocols. It can handle both frozen and paraffin-embedded samples, but cannot be used for mounting, deparaffinization, rehydration, or antigen retrieval steps. Easily adaptable to a variety of immunostaining protocols, the SNAP i.d.® 2.0 IHC System works with all blocking buffers, antibodies, and visualization methodologies (e.g., fluorescence or colorimetric).

Reduced handling time and multiple sample capability make this vacuum-driven system ideal for antibody and protocol optimization.

SNAP i.d.® 2.0 system in the Immunohistochemistry workflow



Immunohistochemistry workflow includes blocking, antibody incubations, labeling and wash steps, all of which can be streamlined using the SNAP i.d.®2.0 Protein Detection System for IHC.

SNAP i.d.® 2.0 IHC Key Features

- Flexibility of multiple slide configurations enables the processing of 1 to 24 slides at a time
- Compatible with standard IHC slides and protocols
- Compatible with diverse tissue preparations including formalin-fixed or fresh frozen samples
- Intuitive format
- Incorporates blocking, washing, and antibody incubation and labeling steps
- Systematizes handling multiple slides without the cost of automation
- Test tracker feature on frame cover helps keep track of IHC steps

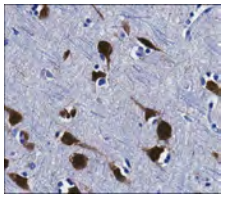
How does the SNAP i.d.®2.0 Protein Detection System for IHC work?

With two individually controlled sides, the system base allows for independent, vacuum-driven processing of either one or two IHC frames. IHC frames have covers and can be removed from the base for extended incubation (one hour to overnight) at 4 °C. Covered frames can be stacked for off-line processing. Each of the IHC frames can process between 1 to 12 glass slides through independent vacuum ports. Empty ports can be plugged when not in use. Each slide holder has an injection/recovery port that enables the manual addition, as well as the removal and recovery, of small volumes of antibodies (greater than 75% antibody recovery) or reagents; reagents can also be flushed using the vacuum feature if conservation is not a priority.

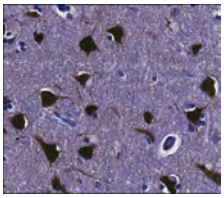
Comparable performance to traditional methods, even in archival tissue

The SNAP i.d.® 2.0 IHC System produces comparable staining to traditional protocols even in archival tissue. The example shows NeuN signal in human brain, localized as expected to neuronal nuclei. Despite processing 12 slides in parallel and shortening the handling time and protocol, the staining is robust and consistent with no blotchy artifacts that sometimes plague autostainers. There is no apparent tissue degradation as compared with traditional protocols. Classic histological stains such as hematoxylin can be applied using the same system.

SNAP i.d.® IHC System



Manual IHC



Detection of NeuN in human cerebral cortex (FFPE): SNAP i.d.®2.0 IHC System (left) vs. standard manual IHC protocol (right). Anti-NeuN (Cat. No. MAB377, 1:2,000) was used to stain sections of human cerebral cortex using the protocols described in the previous figure.

Reli(Ab)le™ Antibodies for IHC

Reliable results depend on reliable reagents. When you work with reliable antibodies, you can work efficiently and effectively. We guarantee performance because we manufacture performance. We are committed to manufacturing antibodies that perform as specified and as anticipated. Our quality starts at inception and carries through manufacturing, production and distribution, into the lab and onto the bench. From start to finish, we build a platform for reliable performance that provides you with the ultimate confidence in your findings.

Ordering Information

Description	Qty	Cat. No.
SNAP i.d.® 2.0 IHC Systems		
SNAP i.d.® 2.0 Protein Detection System - Single IHC		SNAP2IHC
SNAP i.d.® 2.0 Protein Detection System - Double IHC		SNAP2IHC2
SNAP i.d.® 2.0 Consumables		
SNAP i.d.® 2.0 IHC Frame	1 EA	SNAP2FRIHC
SNAP i.d.® 2.0 IHC Slide Holders	24/pk	SNAP2SH
Popular IHC Antibodies		
Anti-Actin Antibody, clone C4	100 µL	MAB1501
Anti-APP A4 Antibody, a.a. 66-81 of APP {NT}, clone 22C11	50 µg	MAB348

Description	Qty	Cat. No.
Anti-Choline Acetyltransferase Antibody	500 µL	AB144P
Anti-GAD67 Antibody, clone 1G10.2	100 µg	MAB5406
Anti-Microtubule-Associated Protein 2 (MAP2) Antibody	100 µL	AB5622
Anti-NeuN Antibody, clone A60	500 µg	MAB377
Anti-NG2 Chondroitin Sulfate Proteoglycan Antibody	100 µg	AB5320
Anti-Olig-2 Antibody	100 µL	AB9610
Anti-Sox9 Antibody	100 µg	AB5535
Anti-Tyrosine Hydroxylase Antibody	100 µL	AB152

Water Purification Systems

Milli-Q® Integral
Simplicity®
Milli-Q® Direct

Water is the most commonly used solvent in laboratories and constitutes often more than 99% of the mass of solutions used in experimentations. The quality of water used in the lab is therefore critical for the success of the tests performed. Ultrapure water is also easily contaminated by materials extracted from containers such as sodium and silica from glass, plasticizers and ions from polymeric materials (for instance, phthalate esters from PVC pipes, fluoride from PTFE pipes) and metallic ions from metallic containers. This is the reason why, in order to minimize the risks of contamination and the experimental variability that can be caused by these contaminants, ultrapure water should be produced just before usage and used at once for glassware final rinsing or the preparation of solutions.

Merck Millpore has developed a range of solutions adapted to the needs of scientists using RNase- & DNase-free water, and water for cell analysis applications (e.g. cell culture). Water quality is an important factor for serum-free culture media preparation. Different cell lines show different sensitivities to the various water types used. However, regardless of cell type, ultrapure water always resulted in the best cell growth.

Milli-Q® Integral system water specifications

Integrated pure and ultrapure water at your fingertips. For scientists who work with a variety of applications requiring both pure (Type 2) and ultrapure (Type 1) water, the Milli-Q® Integral water purification system provides the perfect solution. The system's comprehensive and optimized sequence of water purification and monitoring technologies allows both pure and ultrapure water to be produced directly from potable tap water - in a single unit. Milli-Q® Integral system users gain in convenience and also save on capital expense and lab space.

Ultrapure (Type 1) water quality

Milli-Q® water (sourced from a Q-POD® unit)

Parameter	Value	Unit
Resistivity	18.2	MΩ.cm @ 25 °C
TOC	< 5	ppb (µg/l)
Bacteria	< 0.1 (*)	cfu / ml
Particulates > 0.2 µm	< 1 (*)	Particulates / ml
Pyrogens (Endotoxins)	< 0.001 (**)	EU / ml
RNases*	< 0.01 (**)	ng / ml
DNases*	< 4 (**)	pg / µl

(*) With Millipak® filter with Millipore Express® membrane or BioPak® filter as Application Pak
(**) With BioPak® filter as Application Pak

The Milli-Q® Integral system is designed to produce ultrapure water in agreement with the quantitative specifications of Type I water as described in ISO® 3696, ASTM® D1193, and of EP and USP Purified Water, as well as the CLSI® - CLRW. A compliance report, with test details, is available upon request.

Pure (Type 2) water quality

Elix® water (at Elix® module outlet)

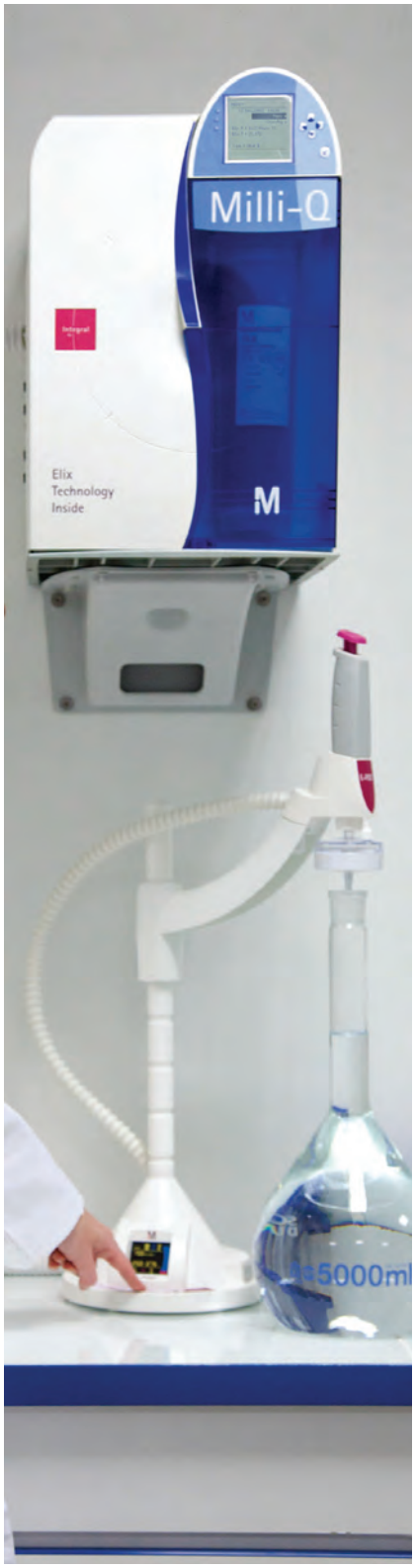
Parameter	Value	Unit
Resistivity	> 5	MΩ.cm @ 25° C
TOC	< 30	ppb (µg/l)

Note: If pure water is sourced from an E-POD®, the following water quality speciications are achieved:

Parameter	Value	Unit
Bacteria	< 0.1 (*)	cfu / ml
Particulates > 0.2 µm	< 1 (*)	Particulates / ml
Pyrogens (Endotoxins)	< 0.001 (**)	EU / ml
RNases*	< 0.01 (**)	ng / ml
DNases*	< 4 (**)	pg / µl

(*) With Millipak® filter with Millipore Express® membrane or BioPak® filter as Application Pak
(**) With BioPak® filter as Application Pak

Resistivity, TOC, and bacteria levels match the requirements of Type 2 water as described in ISO® 3696, ASTM® D1193 (Type II resistivity, TOC, HBC Table I specification), and purifi ed water as described in USP, EP. A compliance report, with test details, is available upon request.



Advantages	Applications
• Type 2 (Pure) and Type 1 (Uitrapure) water directly from tap water • Patented Elix® technology for consistent pure water production • Convenience and flexibility in water delivery with up to 3 Points of Delivery (POD) • Application-specifie water qualities through customized final polishers • Automatic or manual delivery of pressurized Type 2 and Type1 water up to 2 L/min • Installation options adapted to lab space: under bench, above bench or wall-mounted • Complete quality control with advanced resistivity and TOC monitoring in compliance with USP § 643 and § 645 • Full calibration and qualification support for compliance with requirements ofvalidated environments such as cGMP and GLP	• Chromatography: HPLC, UPLC, LC-MS and GC • Elemental analysis: M, GFM, ICP-MS, ICP-OES and ILC • Cell culture • Molecular biology and biochemistry PCR, DNA sequencing, DNA microarray, electrophoresis and blotting • Analytical techniques with sensitivity at ppm level or above such as pH measure and Kjeldahl test • Chemical synthesis: reagents preparation • Staining solutions preparation for histology • Instrument feed for clinical analyzers, weatherometers, glass-ware washing machines, autoclaves, stability test chambers and hydrogen generators

■ Simplicity® Water Purification Systems

Ultrapure water on demand wherever you need it! Choose the solution that's right for you

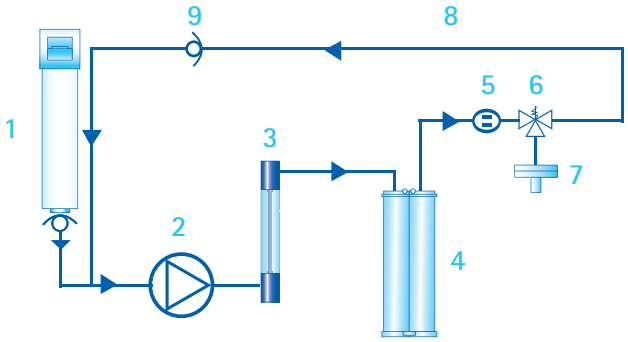
Compact, portable Simplicity water purification systems produce ultrapure water on demand where you really need it: on your lab bench where you perform your experiments. Simplicity systems produce ultrapure water on demand using pure water from the removable 2-liter integrated reservoir.

▶ Specifications

Ultrapure (Type I) Product Water Quality*	Simplicity Systems
Resistivity	18.2 MΩ·cm@ 25 °C
Instant flow rate (with Application Pak final filter)	> 0.5 l/min
TOC (w/o 185/254 nm UV lamp)	< 15 ppb
TOC (with 185/254 nm UV lamp)	< 5 ppb
Particulates (size > 0.22 µm)**	< 1 particulate/ml
Bacteria**	< 0.1 cfu/ml
Endotoxin (pyrogens)***	< 0.001 EU/ml
RNases***	< 0.01 ng/ml
DNases***	< 4 pg/µl

* In regular operating conditions
** With SimFilter (0. 05 µm) end filter or with BioPak® ultrafil-tration cartridge as final polisher
*** Only with BioPak ultrafiltration cartridge as final polisher

▶ Simplicity Systems Water Purification Pathway



1. 2-Liter Removable Reservoir
2. Distribution Pump
3. UV Lamp 185/254 nm (UV System)
4. SimpliPak 1, 2 or 3 Cartridge
5. Product R esistivity Cell
6. Point-of-Use (POU) Solenoid Valve
7. Final Filter / Application Pak
8. Recirculation Loop
9. Check valve

Select an Application Pak to get the best adapted media for your research: BioPack®, VOC-Pack™, EDS-Pak™, LC-Pak™, Millipak® polishers.

Application Pak range

BioPak® Polisher

Pyrogen-free and nuclease-free water

VOC-Pak™ Polisher

Water for volatile organic compounds analysis

EDS-Pak® Polisher

Water for endocrine disrupters experiments

LC-Pak™ Polisher

Water for ultratrace organic analysis

Millipak® Polisher

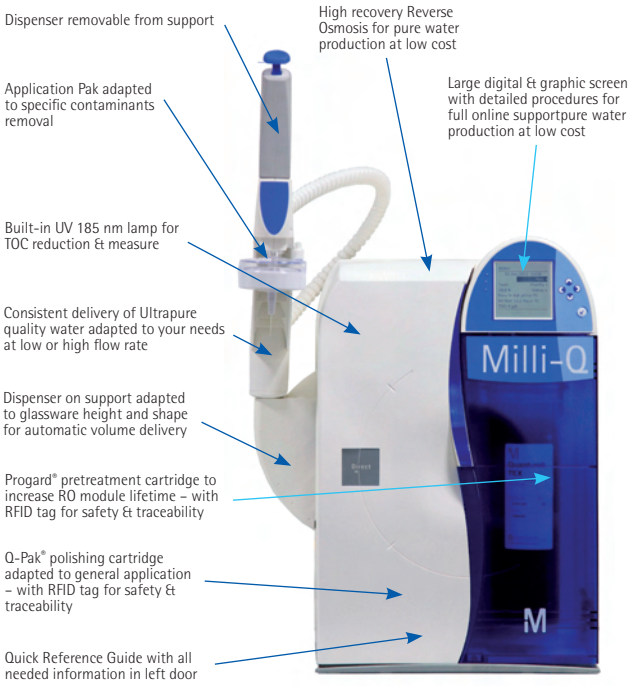
Bacteria-free and particulate-free water

Milli-Q® Direct Water Purification System

Pure & ultrapure water directly from tap water

The Milli-Q® Direct is designed as a single water system which produces pure and ultrapure water directly from tap water.
The system:

- exceeds the requirements of the most demanding norms
- provides manual and volumetric water dispense at low and high flow rate
- has a low footprint: wall-or bench-integrated installation
- allows optimized global costs



Type 1 Water Delivery

Parameter	Value & Unit
Manual dispense flow rate	Adjustable between 50 and 2000 ml/min
Automatic dispense volume	100 ml, then 250 ml to 5 l by 250 ml increments; 5 l to 60 l by 1 l increments
Volumetric dispense accuracy 3	3% for volumes between 250 ml and 60 l
Volumetric dispense dispersion	CV < 3% for volumes between 250 ml and 60 l

Type 3 Water production and delivery

Parameter	Value & Unit
Production Flow Rate	8 l/hour (Milli-Q® Direct 8) 16 l/ hour (Milli-Q® Direct 16)
Delivery Flow rate	From tap: up to 2.5 l/min From optional pump: up to 15 l/min at 1 bar

Type 1 Product Water Quality

Parameter	Value & Unit
Resistivity*	18.2 MΩ.cm at 25 °C
TOC**	≤ 5 ppb (µg/l)
Bacteria ***	< 0.1 cfu/ml
Pyrogens (endotoxins)****	< 0.001 EU/ml (pyrogen-free)
RNases****	< 0.01 ng/ml (RNase-free)
DNases****	< 4 pg/µl (DNase-free)

* Resistivity can be displayed temperature-compensated at 25 °C or nontemperature-compensated as required by USP
** TOC specs - Test Conditions: Milli-Q® Direct System equipped with Progard® T3 pretreatment pack and Q-Pak® TEX polishing cartridge and with feed (tap) water quality within specifications. Product water quality may vary due to local feed water conditions.
*** Results with Millipak® Express 40 or BioPak® final polisher in place
**** Results with BioPak® final polisher in place

Type 3 Water Quality

Parameter	Value & Unit
Ions rejection	97 to 98% with new RO cartridge
Organics Rejection	> 99% for MW > 200 Dalton
Particulates & Bacteria Rejection	> 99%

To Place an Order or Receive Technical Assistance

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