Fast, Accurate, Innovative Cell Counting & Analysis

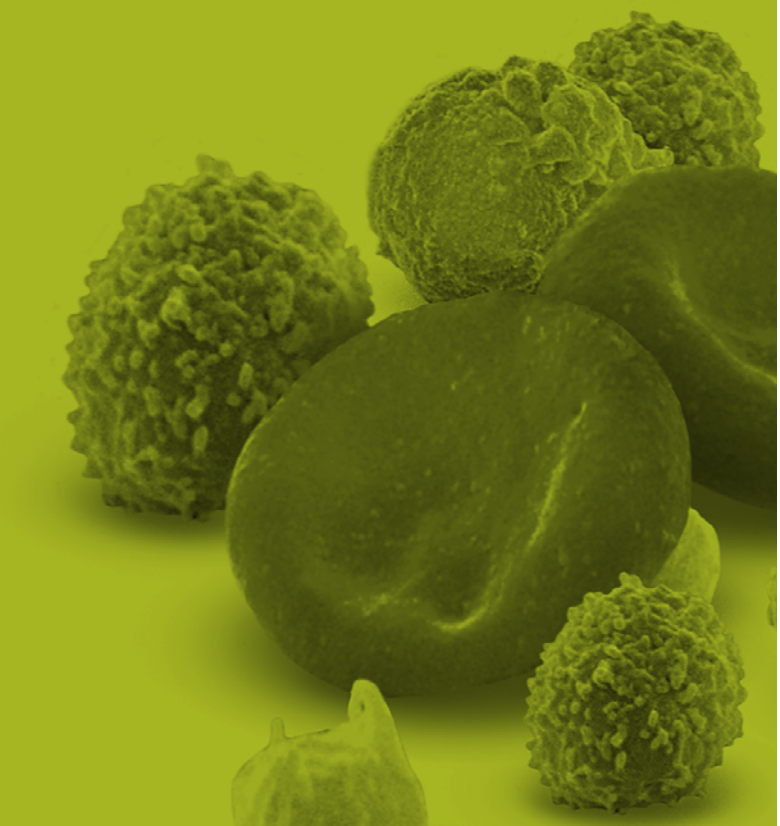
Discover the Muse® Cell Analyzer and Scepter™ Cell Counter

Merck Millipore is a division of MERCK

Cell Counting and Analysis

When you have easy access to precise cell counts and highly quantitative analysis of cell health parameters, it's so much easier to validate your experiments with an extra dimension of data. We offer you compact, easy-to-use, highly quantitative solutions with minimal setup and execution time so that your work in the lab is its most productive. With Merck Millipore's innovative Cell Counting and Analysis platform1s, you can be confident in the quality of your data with:

- Simpler solutions
- The most accurate and reliable data
- Smart, intuitive technologies



Muse® Cell Analyzer:
Simple, affordable flow cytometry, right on your benchtop

Sophisticated cell analysis doesn't have to be exclusive, complicated, or costly. With the Muse® Cell Analyzer, you can now achieve highly quantitative results at a fraction of the price, effort, and time. The Muse® Cell Analyzer packs 3-parameter analysis into a compact, easy-to-use benchtop device, making flow cytometry accessible to anyone, anytime. A user-friendly touchscreen interface, intuitive software and optimized assays work to simplify your research.

Scepter™ 2.0 Cell Counter:
Precise, portable cell counting

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.

Table of Contents

Muse® Cell Analyzer

Simple, Affordable Flow Cytometry by Your Side

The Muse® Cell Analyzer is a highly intuitive, compact instrument for the fluorescence-based, 3-parameter analysis of cell populations. 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 size; (footprint of only 8 in x 10 in (20 cm x 25 cm)
- Affordable

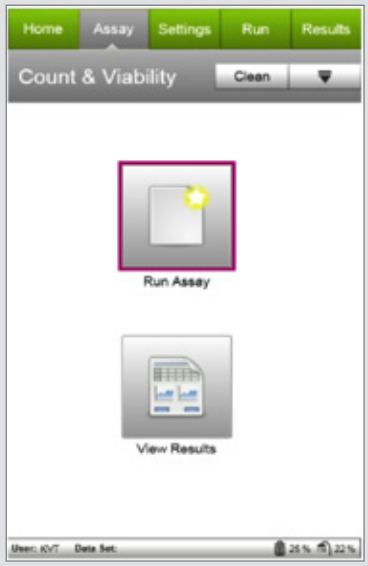
Novel, Miniaturized Flow Technology

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. 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).

The system uses a microcapillary and miniaturized optics, which occupy one-tenth the space of a typical cytometer. This means that the instrument occupies only an 8 in x 10 in (20 cm x 25 cm) footprint. A green diode laser is used for excitation, and a uniquely designed series of retro-reflective lenses provide maximum light capture and sensitivity.

Highly Intuitive Touchscreen Interface

Muse® features a highly intuitive touchscreen interface that allows simple step-by-step operation, so easy that no flow expertise is required to run assays. The touchscreen prompts you through simple on-screen instructions and guides you though sample loading to simple setting adjustments to results—in just a few steps!



Load Sample

Optimized, Simple-Prep Assays and Software Modules

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.

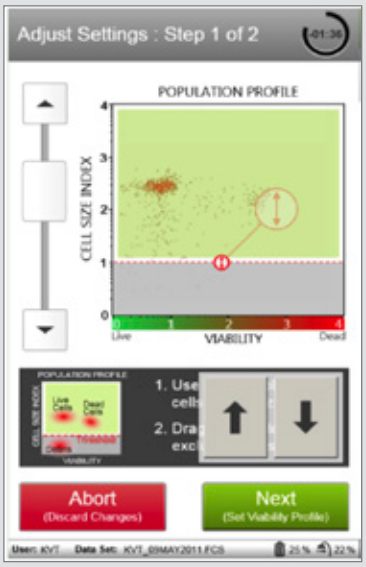
Just prepare your sample with Muse® reagents, load on the instrument, and follow the easy, guided menus on the touchscreen to get your results. Results are displayed in both graphical and statistical formats specific to each application, making analysis unambiguous. Spend less time with experimental setup, avoid reagent waste and save money. 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.

Muse® Assays

Choose from a broad range of Muse® Assays for interrogating multiple aspects of cell biology:

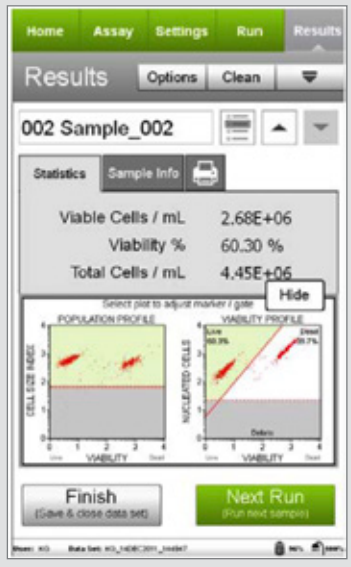
- Count & viability
- Apoptosis
- Cell cycle
- Autophagy
- Cell proliferation
- Cell signaling
- DNA damage
- Immunology

We are continually releasing new Muse® assay modules and kits. Please visit **www.merckmilllipore.com/muse** for the most up-to-date listing of Muse® Assays. New assay software modules can be downloaded free of charge from the website.



Adjust Settings

Acquire



Results!

Muse® Assays for Cell Health Analysis

In addition to using cell health assessment for elucidating disease mechanisms and therapeutic discovery, monitoring key indicators of cell health and performance helps establish uniform standards of cellular performance across long-term research studies. Knowing the performance profile of your cells prior to running your bioassay can mean the difference between valid assay results and wasted reagents, lost time and discarded data. Get truly quantitative, accurate data on the health of your cells. Make the Muse® Cell Analyzer and Muse® Cell Health Assays your everyday partners in cell health assessment.

Featured Kits

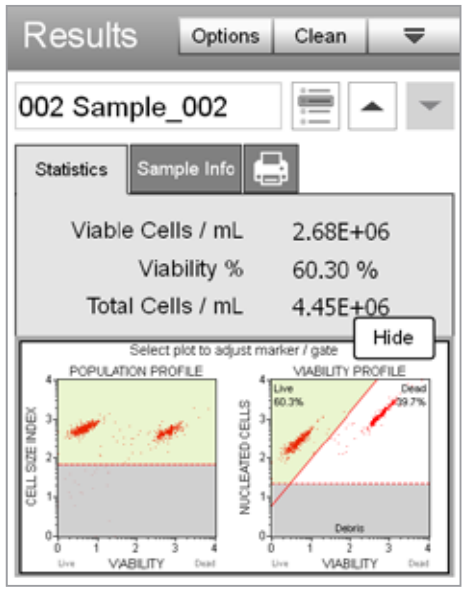
Muse® Count and Viability Kit

(Cat. No MCH100102)

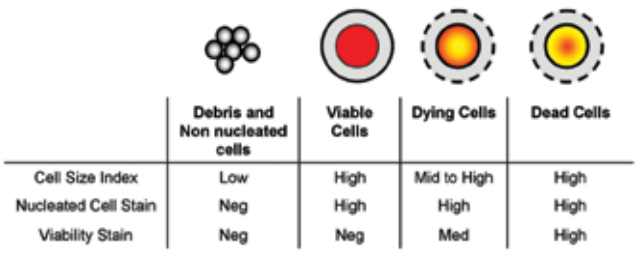
This simple, linear assay rapidly provides cell concentration and viability information. The assay uses a premixed reagent composed of two DNA-intercalating fluorescent dyes. One of the dyes is membrane-permeant and stains all cells with a nucleus. The second dye only stains dying or dead cells, whose 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, resulting in both accurate cell concentration and viability data. Stained samples are then analyzed on the Muse® Cell Analyzer. The use of dual fluorescent probes that clearly identify all nucleated cells, live and dead, allows for greater sensitivity and accuracy compared to colorimetric methods.

The following data outputs are shown:

- Viable Cell Count (cells/mL)
- Percentage of Viable Cells
- Total Cell Count (cells/mL)



Data output for the Muse® Count and Viability Assay. Healthy Jurkat cells were mixed with heat-killed Jurkat cells and stained with Muse® Count & Viability Reagent, and then analyzed on the Muse® Cell Analyzer. Data output include summary data (not shown) and optional dot plots (shown here). Reported statistics include viable cells/mL, % viability, and the total cells/mL. The left hand dotplot shows viability vs cell size; the right hand plot shows viability vs nucleated cells.



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

Anticipated staining pattern of the four cell subpopulations analyzed usingthe Muse® Count and Viability Assay.

ALSO AVAILABLE: Kits for analysis of Oxidative Stress, Nitric Oxide and Ki67 Proliferation. Please see **www.merckmillipore.com/muse** for further information.

Muse® Cell Cycle Assay

(Cat. No MCH100106)

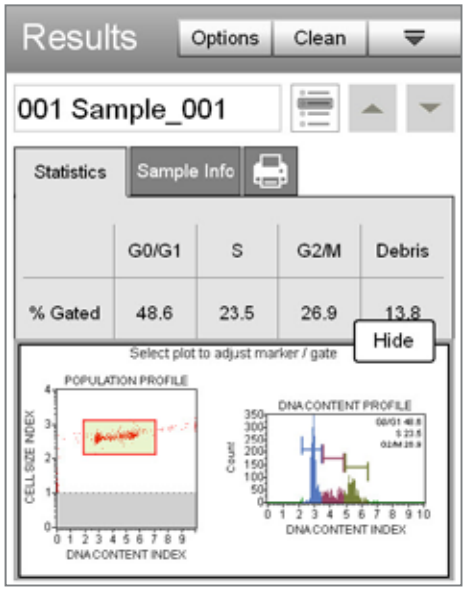
This assay allows for the facile, rapid, and quantitative measurements of percentage of cells in the G0/G1, S, and G2/M phases of cell cycle on the Muse® Cell Analyzer. Unlike traditional cell cycle analysis, which has traditionally required complicated instrumentation and training, the Muse® system enables users to easily determine cell cycle distribution on their benchtops. 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.

Fast, Easy Protocol

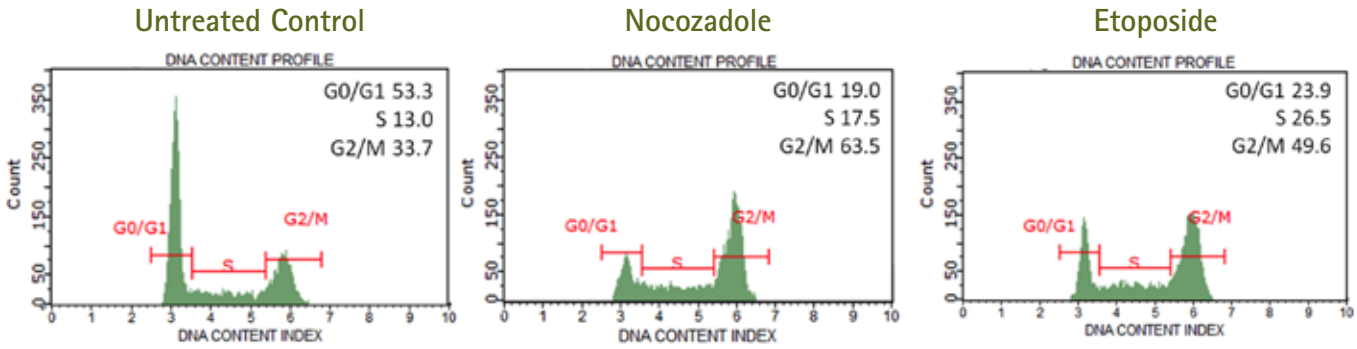
Simply fix in ethanol and incubate with Muse® Cell Cycle Reagent for 30 minutes. After the samples are analyzed on the Muse® instrument, the percentages of populations are automatically displayed, along with a histogram with three markers to demarcate the G0/G1, S, and G2/M cell cycle phases.

Cell populations identified:

- Cells in the G0/G1 phase
- Cells in the S phase
- Cells in the G2/M phase



Data output for the Muse® Cell Cycle Assay. Jurkat cells were stained with the Muse® Cell Cycle Reagent and analyzed on the Muse® Cell Analyzer. Data output include summary data (not shown here), and a dotplot and histogram showing the cell cycle distribution.



Analyzing the impact of cell cycle–disrupting compounds with the Muse® Cell Analyzer. The Muse® Cell Cycle Assay can be used for a variety of cellular treatment conditions and to study the impact of cell cycle disrupting compounds. Nocodazole, a microtubule disrupter, leads to cell cycle arrest in G2/M phase; etoposide, a known anti-cancer compound, also causes G2/M arrest.

Muse® Autophagy LC3-Antibody Based Kit

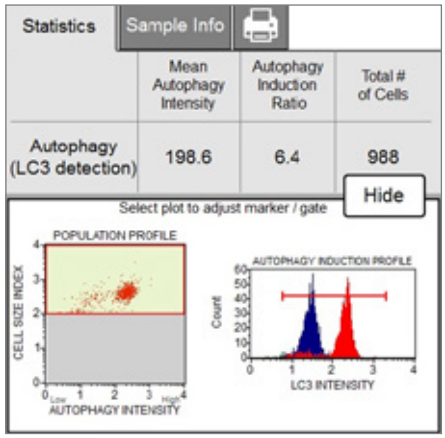
(Cat. No. MCH200109)

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 using flow cytometry. 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 or imaging. 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 antibody specificity/species cross reactivity is for human, but is also validated for mouse, rat, and hamster via western blot.

Data generated using the Muse® Cell Analyzer along with the corresponding Muse® software module provide statistical values measuring:

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



HeLa cells were either starved for 4 hours to induce autophagy or kept under fed conditions, and samples were prepared using the reagents in the Muse® Autophagy LC-3 Antibody Based Kit. The figure above shows the histogram plot comparing the control versus the target sample. Results are also displayed on the Muse® instrument in a summary page format (not shown). Here, the mean autophagy intensity for each sample is determined and the autophagy induction ratio is then calculated based on the ratio between the target sample fluorescence versus the control sample. In this cell population, there is a 6.4-fold change between the control sample (e.g. no autophagy in blue) when compared to the starved sample (e.g. induced autophagy in red), indicating the presence of autophagy.

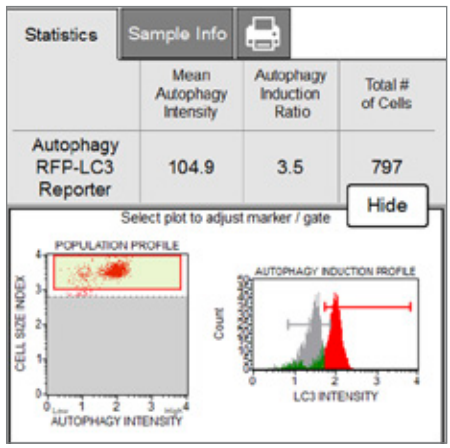
Muse® RFP-LC3 Reporter Autophagy Assay

(Cat. No. MCH200110)

Quantitate autophagy in single cells by tracking the autophagy protein, LC3, using this rapid, simple assay. This kit includes an mRFP-LC3 reporter cell line (stably transfected U20S human osteosarcoma cells that constitutively produce high levels of LC3) for comparative studies. 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. A monomeric RFP is used as a reporter to facilitate the translocation of the fusion protein.

Data generated using the Muse® Cell Analyzer along with the corresponding Muse® software module provide statistical values measuring:

- Mean Autophagy Value (for both control and test samples)
- Autophagy Induction Ratio (test sample fluorescence relative to control)
- Percentage of cells with increased autophagy (test sample versus control)



Data output for Muse® RFP-LC3 Reporter Autophagy Assay. The RFP-LC3 reporter cell line was either starved for 4 hours to induce autophagy or kept under fed conditions, and then treated with autophagy reagents A and B (during sample preparation steps). Here, the mean autophagy intensity for each sample is determined and the autophagy induction ratio is then calculated based on the ratio between the target sample fluorescence versus the control sample. In this cell population, there is a 3.5-fold change between the control sample (e.g. no autophagy in gray) when compared to the starved sample (e.g. induced autophagy in red), indicating the presence of autophagy. Moreover, the percentage of increased autophagy is calculated between control and test samples, where it is calculated at 85.4% (not shown).

Muse® Assays for Apoptosis Analysis

The degree of apoptosis in a cell population is an important parameter that contributes to a comprehensive picture of cell health. The assessment of cellular apoptosis has been limited due to the requirements for expensive and complicated instrument platforms, expertise and improved analytical methods that provide rapid, robust and reproducible apoptosis data. The Muse® Cell Analyzer delivers access to these improvements, facilitating apoptosis monitoring and thereby enabling the efficient, daily execution of cellular research.

Muse® apoptosis kits allow the study of early, mid, and late apoptosis, and also a pan-caspase assay for broad apoptosis activity:

- Muse® Annexin V and Dead Cell (mid-late apoptosis)
- Muse® Mitopotential (early apoptosis)
- Muse® Caspase-3/7 (late apoptosis)
- Muse® MultiCaspase (multiple apoptosis indicators)

Featured Kits

Muse® MitoPotential Assay

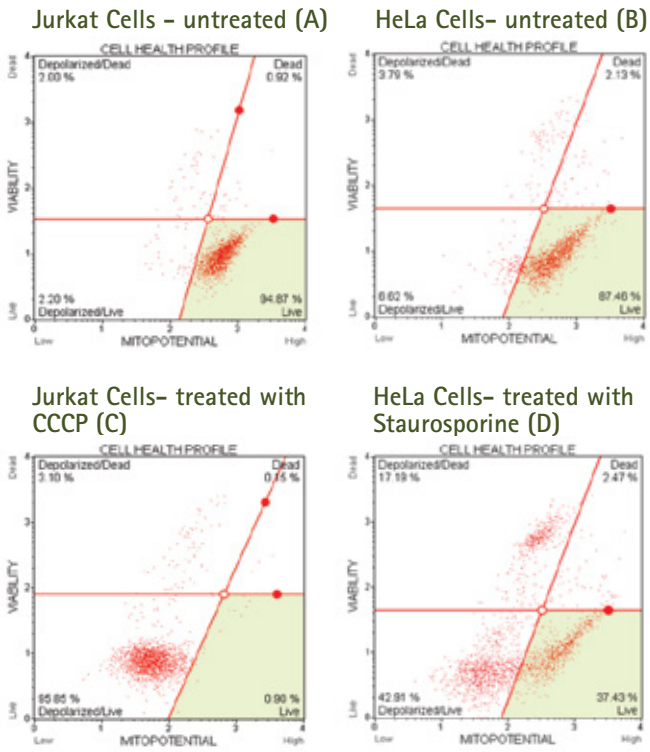
(Cat. No. MCH100105)

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.

Four populations of cells can be distinguished in the assay:

- Live cells with intact mitochondrial membrane: MitoPotential(+) and 7-AAD(-)
- Live cells with depolarized mitochondrial membrane: MitoPotential(-) and 7-AAD(-)
- Dead cells with depolarized mitochondrial membrane: MitoPotential(-) and 7-AAD(+)
- Dead cells with intact mitochondrial membrane: MitoPotential(+) and 7-AAD(+)



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).

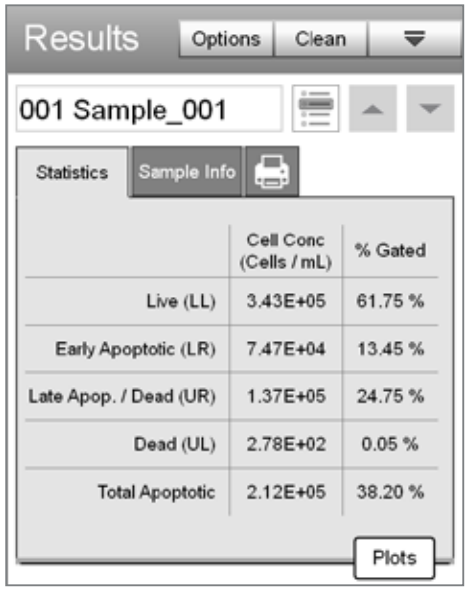
Muse® Annexin V & Dead Cell Assay

(Cat. No. MCH100105)

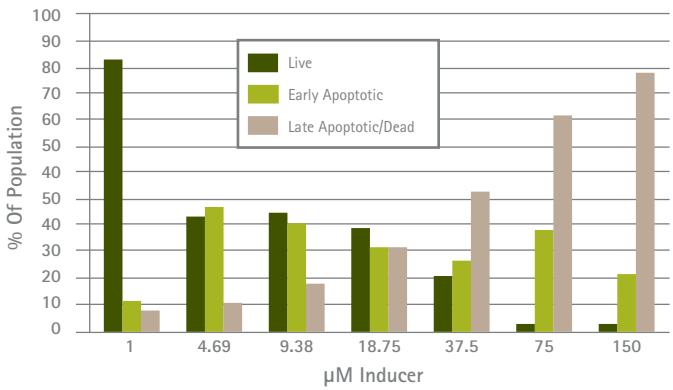
This versatile assay can be used to assess health of both adherent and suspension cells under multiple treatment conditions and generate dose-response data on cells treated with apoptosis inducers. The assay is based on the binding of Annexin V to phosphatidylserine (PS) on the surface of apoptotic cells. 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.

The assay can thus distinguish four populations:

- Non-apoptotic cells: Annexin V (-) and 7-AAD (-)
- Early apoptotic cells: Annexin V (+) and 7-AAD (-)
- Late stage apoptotic and dead cells: Annexin V (+) and 7-AAD (+)
- Mostly nuclear debris: Annexin V (-) and 7-AAD (+)



Data output from the Muse® Annexin V and Dead Cell Assay. Jurkat cells were treated with the apoptosis inducer gambogic acid and analyzed with the Muse® Annexin V and Dead Cell Assay. Data output include summary statistics for the four populations (shown here) as well as optional dotplots (not shown).



Response of Jurkat cells treated with increasing concentrations of gambogic acid (apoptosis inducer) and analyzed using the Muse® Annexin V and Dead Cell Assay.

Muse® Caspase-3/7 Assay

(Cat. No. MCH100108)

Caspase-3 and caspase-7 are "executioner caspases" that are activated downstream in the apoptosis cascade by a sequence of intrinsic or extrinsic signals. Once activated, these enzymes cause degradation of many key cellular proteins and influence chromatin condensation and DNA damage during apoptosis. Activation of caspase-3/7 is thus a hallmark and confirmation of the apoptotic process.

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.

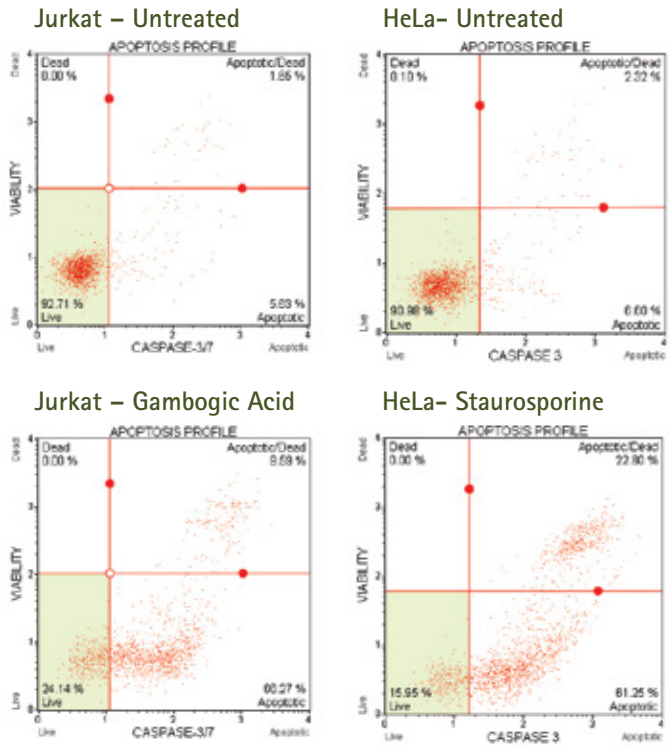
The kit includes:

1. The novel, fluorogenic Muse® Caspase-3/7 reagent for detecting caspase-3/7 activity
2. 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.

Four populations of cells can be distinguished in the assay:

- Live cells: caspase-3/7(-) and 7-AAD(-)
- Mid-apoptotic cells exhibiting caspase-3/7 activity: caspase 3/7(+) and 7-AAD(-)
- Late apoptotic/dead cells: caspase-3/7(+) and 7-AAD(+)
- Dead cells: caspase-3/7(-) and 7-AAD(+)



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

We are continually releasing new Muse® assay modules and kits. Please visit **www.merckmilllipore.com/muse** for the most up-to-date listing of Muse® Assays. New assay software modules can be downloaded free of charge from the website.

Muse® MultiCaspase Assay

(Cat. No. MCH100109)

This assay detects the activity of caspases 1, 3, 4, 5, 6, 7, 8 and 9, which have multiple roles, in addition to carrying out apoptosis. The assay simultaneously determines the percentage and concentration of cells with caspase activity, in combination with a dead cell dye.

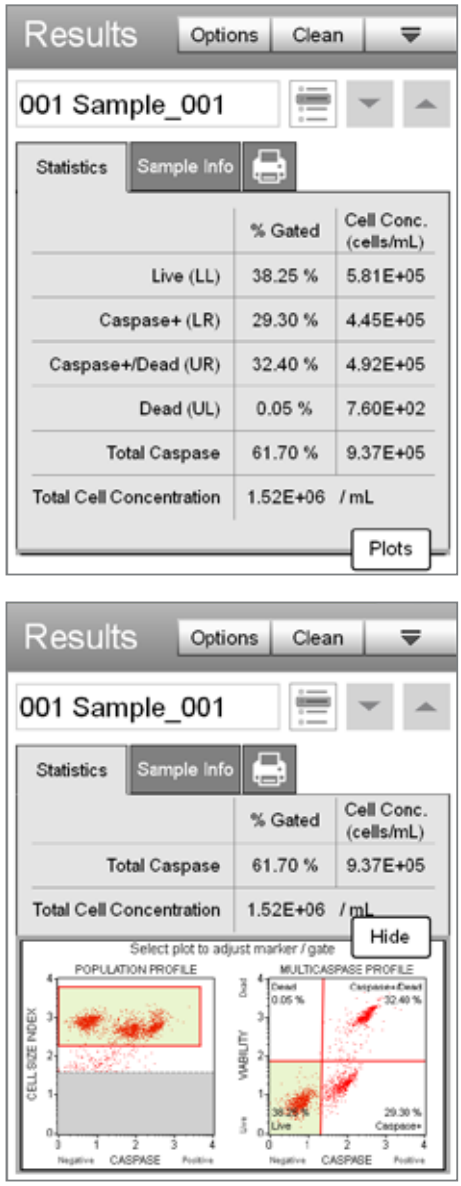
The kit includes:

1. A fluorogenic, derivatized VAD-peptide that can detect the activity of multiple caspases
2. 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.

Four populations of cells can be distinguished in the assay:

- Live cells: caspase (-) and 7-AAD(-)
- Cells exhibiting pan caspase activity: caspase(+) and 7-AAD(-)
- Late caspase active/dead cells: caspase(+) and 7-AAD(+)
- Dead cells: caspase(-) and 7-AAD(+)



Data output for the Muse® MultiCaspase Assay. Jurkat cells were treated with staurosporine, stained with the Muse® MultiCaspase Kit and analyzed on the Muse® Cell Analyzer. The summary data with statistics (top) show the percentages and the concentration (cells/mL) for the gated events in each quadrant, as well as the percentage and concentration of total caspase-positive cells. Data is also displayed as dot plots (bottom). The first dotplot (left) shows cell size index vs. caspase activity and the second plot (right) shows viability vs. caspase activity.

Muse® Assays for Cell Signaling Analysis

Current methods for monitoring cell signaling, such as Western blotting, ELISA, bead-based assays and flow cytometry, all have advantages and limitations. Population analyses (such as Westerns, ELISAs or bead-based assays) are quantitative, but do not allow discrimination at the single cell level, masking true response. Traditional flow cytometry can provide highly quantitative data on single cells, but expertise, costly instrumentation or access to a core facility may stand in the way of routine use in signaling research. The Muse® Cell Analyzer provides the highly quantitative, reproducible, single cell-level results of flow cytometry in a compact, benchtop platform requiring little sample preparation and minimal expertise.

Activation Dual Detection Kits

These simple, yet precise, assays for study cell signaling pathways each 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

Choose from multiple Muse® kits to assess the activation of key cell signaling pathways:

- Muse® H2A.X Activation Dual Detection Kit (Cat. No. MCH200101)
- Muse® EGFR-RTK Activation Dual Detection Kit (Cat. No. MCH200102)
- Muse® PI3K Activation Dual Detection Kit (Cat. No. MCH200103)
- Muse® MAPK Activation Dual Detection Kit (Cat. No. MCH200104)
- Muse® Bcl-2 Activation Dual Detection Kit (Cat. No. MCH200105)
- Muse® PI3K/MAPK Activation Dual Detection Kit (Cat. No, MCH200108)
- Muse® Multi Color DNA Damage Kit (H2A.X/ATM) (Cat No. MCH200110)

We are continually releasing new Muse® assay modules and kits. Please visit **www.merckmilllipore.com/muse** for the most up-to-date listing of Muse® Assays. New assay software modules can be downloaded free of charge from the website.

Featured Kits

Muse® H2A.X Activation Dual Detection Assay

(Cat. No. MCH200101)

This assay allows the researcher to monitor and accurately measure phospho-specific Histone H2A.X activation in a population of cells. The histone H2A.X resides downstream of the DNA damage kinase signaling cascade. As the level of DNA damage increases, the level of phospho Histone H2A.X (also known as γH2A.X) increases, accumulating at the sites of DNA damage. This accumulation of phospho Histone H2A.X is often used to indicate the level of DNA damage in the cell.

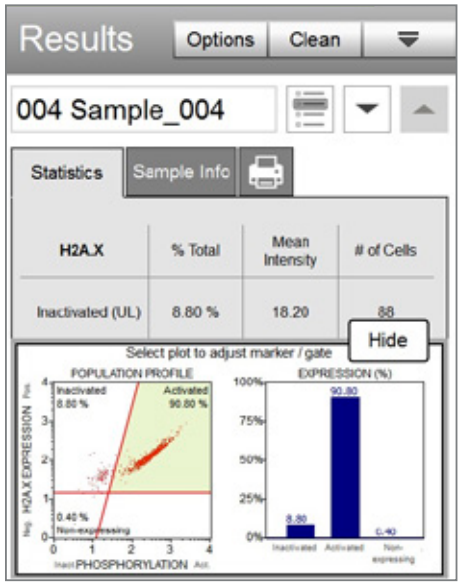
The Muse™ H2A.X Activation Dual Detection Kit includes two directly conjugated antibodies, a phospho-specific anti-phospho-Histone H2A.X (Ser139)-Alexa Fluor®555 and an anti-Histone H2A.X-PECy5 conjugated antibody to measure total levels of Histone H2A.X. This two-color kit is designed to detect the extent of Histone H2A.X pathway activation by measuring H2A.X phosphorylation relative to the total H2A.X expression in any given cell population.

Muse® PI3K Activation Dual Detection Kit

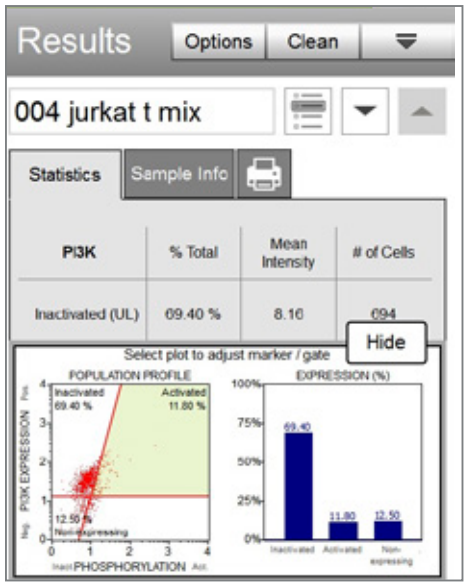
(Cat. No. MCH200103)

Akt/PKB is a Ser/Thr kinase and a major known effector of the PI3 Kinase pathway. It is involved in multiple signaling pathways regulating metabolism, apoptosis, cell cycle control, angiogenesis, differentiation, cell growth, proliferation and more.

The Muse® PI3K Activation Dual Detection Kit includes two directly conjugated antibodies, a phospho-specific anti-phospho-Akt (Ser473), Alexa Fluor®555 and an anti-Akt, PECy5 conjugated antibody to measure total levels of Akt. Use this two-color kit to measure the extent of Akt phosphorylation relative to the total Akt expression at a single-cell level.



Data output for the Muse® H2A.X Activation Dual Detection Kit. HeLa cells were exposed to 10 µM Etoposide for 24 hours to induce DNA damage, stained with both anti-phospho-Histone H2A.X (Ser139) and anti-Histone H2A.X antibodies in multiplex and analyzed using the Muse® Cell Analyzer. Summary data and statistics (not shown) show the relative percentages for each population as it is calculated within the total cell population. Dot plot and bar graph data are shown here. Cells that express H2A.X correspond to the data in the top two quadrants of the dot plot (inactivated and activated, representing about 99.6% of the total cell population). But of this cell population, 90.8% is activated upon treatment, indicating that DNA damage is present. By presentation of both data sets, the total: phospho ratio can be determined.



Data output for Muse® PI3K Kinase Activation Dual Detection Assay. In Jurkat cells, Akt is constitutively activated. Jurkat cells were exposed to 1 µM wortmannin for 60 minutes at 37°C to inhibit the Akt signaling cascade response, fixed, permeabilized, and then stained with both anti-phospho- Akt (Ser473) and anti-Akt/PKB antibodies in multiplex. Samples were analyzed using the Muse® Cell Analyzer. The statistics captured in this assay show the relative percentages for each subpopulation as it is calculated within the total cell population. Cells which express Akt correspond to the top two quadrants of the dot plot (81.2% of the total cell population). But of this cell population, 69.4% is deactivated upon treatment, attenuating the constitutive activation of the Akt signaling pathway. By presentation of both data sets, the total: phospho ratio can be determined.

Muse® Multi-Color DNA Damage Kit

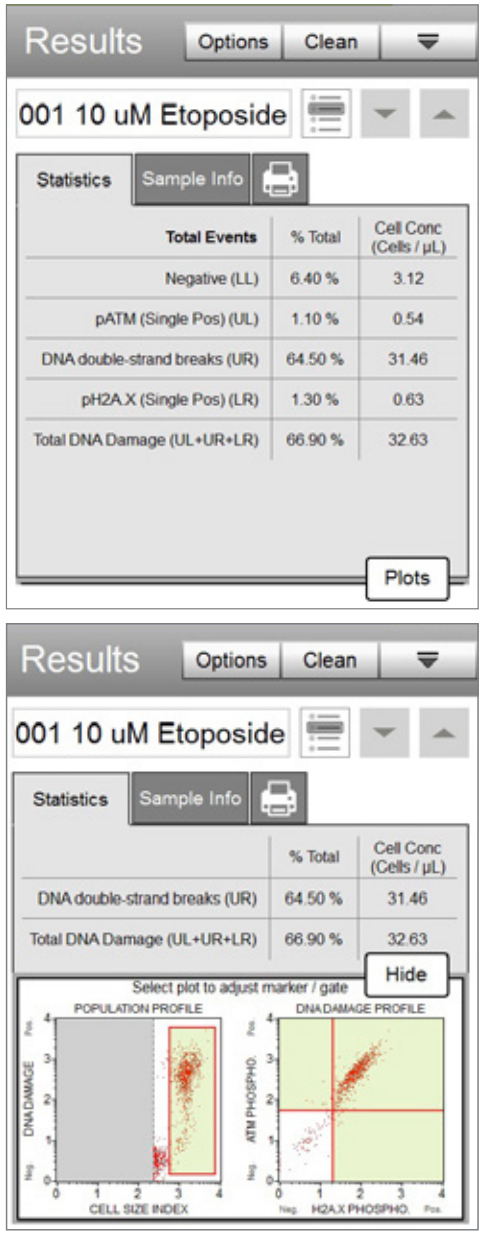
(Cat. No. MCH200107)

This kit provides a quick and easy way to detect the activation of ATM and H2A.X by flow analysis. The kit includes two directly conjugated antibodies, a phospho-specific ATM (Ser1981)-PE and a phospho-specific Histone H2A.X-PECy5 conjugated antibody to measure the extent of DNA damage. Both antibodies are carefully titrated and optimized together to ensure maximal performance when run in multiplex, alleviating the need for any additional optimization. Two-color analysis of ATM and Histone H2A.X activation in multiplex provides more reliable DNA damage detection than measuring either protein on its own.

This kit also contains optimized fixation, permeabilization, and assay buffers to providea complete solution for DNA damage signaling analysis.

Data generated include:

- Percentage of negative cells (no DNA damage)
- Percentage of ATM-activated cells
- Percentage of H2A.X-activated cells
- Percentage of DNA double-strand breaks (dual activation of both ATM and H2A.X)



Data output for Muse® Multi-Color DNA Damage Kit. HeLa cells were exposed to 10 µM Etoposide for 24 hours to induce DNA damage, and then stained with both anti-phospho-Histone H2A.X (Ser139) and anti-phospho-ATM (Ser1981) antibodies in multiplex. Samples were acquired using the Muse® Cell Analyzer. The statistics captured in this assay show the relative percentages for each population as it is calculated within the total cell population (top). Cells which express ATM, H2A.X, or both can be seen by the data on upper left, lower right, and upper right quadrants of the dot plot, respectively (bottom). In this cell population, 64.5% shows co-activation of ATM and H2A.X upon treatment, indicating DNA damage and double-strand breaks are present.

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)

We are continually releasing new Muse® assay modules and kits. Please visit **www.merckmilllipore.com/muse** for the most up-to-date listing of Muse® Assays. New assay software modules can be downloaded free of charge from the website.

Featured Kits

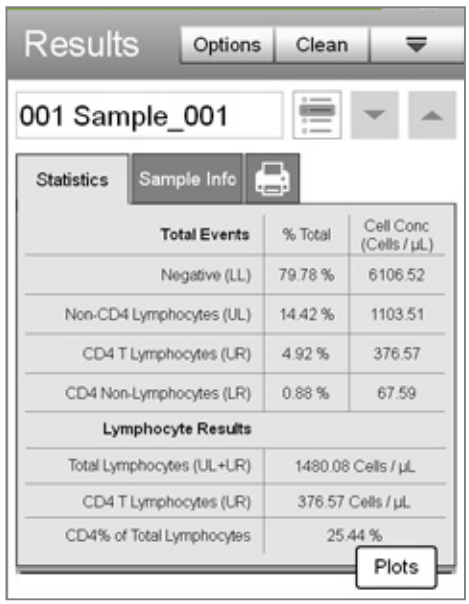
Muse® Human CD4 T Cell Kit

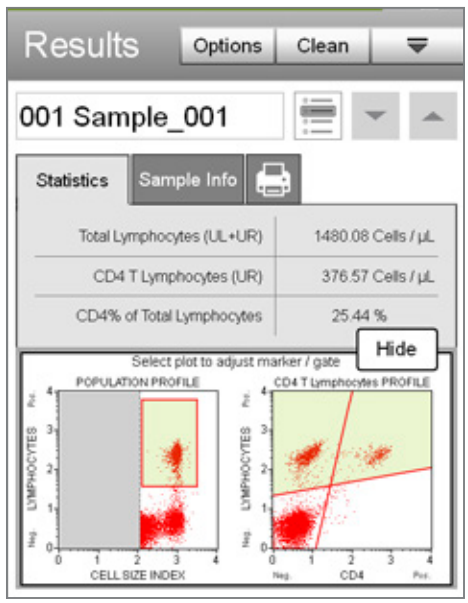
(Cat. No. MIM100101)

Quickly determine the CD4 T-cell count, total lymphocyte count, and CD4 T-cell percent of lymphocytes in human whole blood and peripheral blood mononuclear cell (PBMC) samples. Monocytes also express CD4 but at a lower density, and have no co-expression of the other antibodies present in the anti-lymphocyte cocktail; hence, monocytes can be distinguished from CD4 T cells using this kit. The Muse® CD4 T Cell Assay uses an anti-lymphocyte antibody cocktail that identifies the lymphocyte population, and CD4 antibody that binds to the CD4 cells. Minimal sample preparation is required in this no-wash assay to obtain accurate and precise results. For research use only; not for use in diagnostic procedures.

Data generated include:

- CD4 T-cell concentration in cells/µL
- CD4 T-cell percent of lymphocytes
- Total lymphocyte concentration in cells/µL





Data output for Muse® Human CD4 T Cell Kit. Whole blood was stained with the Muse® Human CD4 T Cell Kit and analyzed on the Muse® Cell Analyzer. The statistics show the percentages and the concentrations (cells/µL) for the results in each quadrant out of the total events (top). The lymphocyte results show the total lymphocyte concentration, the CD4 T-lymphocyte concentration and the CD4 cell percentage of lymphocytes. The first plot in the bottom panel shows lymphocyte count vs. cell size index and a lymphocyte gate, and the second plot shows lymphocyte count vs. CD4 staining.

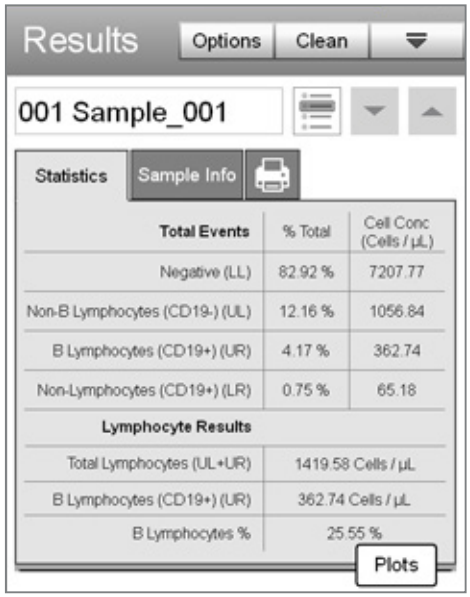
Muse® Human B Cell Kit

(Cat. No. MIM100103)

Determine the B-cell count, total lymphocyte count, and B-cell percent of lymphocytes in human whole blood and peripheral blood mononuclear cell (PBMC) samples. The Muse® Human B Cell Kit detects and identifies lymphocytes and CD19 B lymphocytes in either whole blood or PBMCs. CD19 is considered to be a characteristic B cell marker and therefore commonly used in the routine immunophenotyping of B cells. Minimal sample preparation is required in this no-wash assay to obtain accurate and precise results.

Data generated include:

- B-cell concentration in cells/µL
- B-cell percent of lymphocytes
- Total lymphocyte concentration in cells/µL



Data output for Muse® Human B Cell Kit. Whole blood was stained with the Muse® Human B Cell Kit and analyzed on the Muse® Cell Analyzer. The statistics show the percentages and the concentrations (cells/µL) for the results in each quadrant out of the total events. The lymphocyte results show the total lymphocyte concentration, the B-lymphocyte concentration, and the B-cell percentage of lymphocytes.

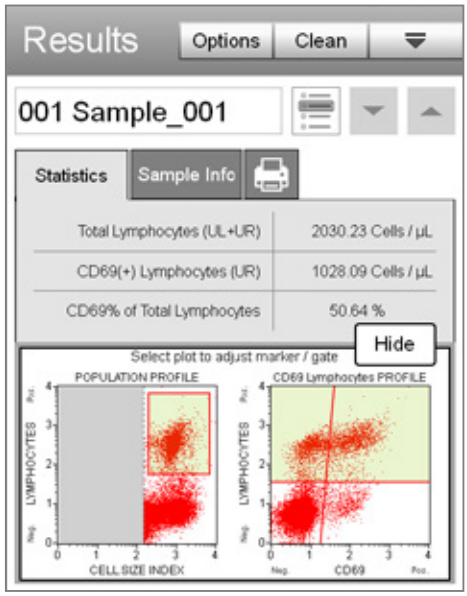
Muse® Human CD69 Lymphocyte Kit for Lymphocyte Activation Studies

(Cat. No. MIM100104)

Determine the CD69(+) lymphocyte count, total lymphocyte count, and CD69 percent of total lymphocytes in human whole blood and peripheral blood mononuclear cell (PBMC) samples. CD69 is an early-activation marker expressed on the surface of activated lymphocytes including T and B lymphocytes and NK cells. The Muse® Human Lymphocyte CD69 Assay uses an anti-lymphocyte cocktail that identifies the total lymphocyte population and a CD69 antibody that binds to the CD69 early activation marker expressed on lymphocytes. The kit also includes an isotype control antibody. Minimal sample preparation is required in this no-wash assay to obtain accurate and precise results.

Data generated include:

- CD69(+) lymphocyte concentration in cells/µL
- CD69 percent of total lymphocytes
- Total lymphocyte concentration in cells/µL



Data output for Muse® Human CD69 Kit. Data output for Muse® Human CD69 Kit. Whole blood was stained with the Muse® Human Lymphocyte CD69 Kit and analyzed on the Muse® Cell Analyzer.

The statistics show the percentages and the concentrations (cells/µL) for the results in each quadrant out of the total events (screen not shown). The lymphocyte results show the total lymphocyte concentration, the CD69(+) lymphocyte concentration, and the CD69 percentage of total lymphocytes. The first plot (left panel) shows cell size index vs. lymphocytes and a lymphocyte gate, and the second plot shows CD69 staining vs. lymphocytes.

Scepter™ 2.0 Portable Cell Counter –

Precise, handheld cell counting

Scepter™ 2.0 is your portable 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.

Scepter™ 2.0 marks the next generation in Scepter™ technology, highlighted by:

Compatibility with More Cell Types

The Scepter™ cell counter is the only one on the market to accurately count particles as small as 3 µm in diameter

Increased Cell Concentration Range

The new 40 µm sensor can count samples with concentrations as high as 1,500,000 cells/mL

Powerful Software for Complex, Effortless Cell Analysis

- Compare sample sets side by side using histogram overlay and multiparametric data table
- Create and save gating templates
- Generate reports, graphs and tables

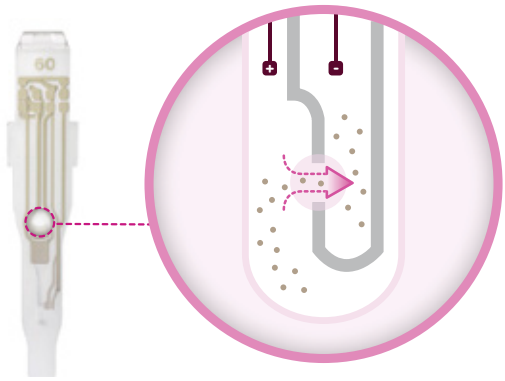
The power of precision

Trust Scepter™ counting with your most valuable samples to get reproducible and reliable counts. The reliability of Scepter™ counting is particularly apparent with smaller cell types. 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.

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. Use the 40 µm sensor for particles between 3 and 17 µm.

- 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.



Particles are detected by Ohm's Law, V=IR. (V=Voltage, I=current, 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	16-17		
MG-63	15-17		
Mouse ES Cell	5-13		
Mesenchymal Stem Cell	15-16		
MRC-5			
NCI-H146	10-13		
NIH 3T3	15		
NTERA2, clone D1	13		
OK	17-18		
PBMCs	7-12		
PC12	9-13		
Primary Astrocytes	7		
Primary Neuronal Cell			
Raji	12-15		
Ramos	11-12		
Rat Dorsal Root Ganglion Cells	7		
Rat Whole Blood	4.6		
Red Blood Cells	5-7		
Rat Neural Stem Cell	11-13		
RAW 264.7	12-15		
RBL	11-13		
RIN-mF5	13-14		
SF9	13		
SH-SY5Y	12		
Sk-Br-3	15-20		
SK-MEL-28	17-19		
SK-N-MC	14-15		
SK-N-SH	14-15		
Splenocytes	7-9		
SW-480	15		
SW-620	13-14		
T84	14-18		
T98G	17		
TF-1	13-14		
U251	16-20		
U2OS	16-19		
U266	12		
U87-Human Glioblastoma cell line	12-14		
U937	11-13		
WI-38	12-15		
Y79	13-14		
Yeast- Pichia Pastoris	5		
Yeast- S.cerevisiae	6		

Recommended based on size

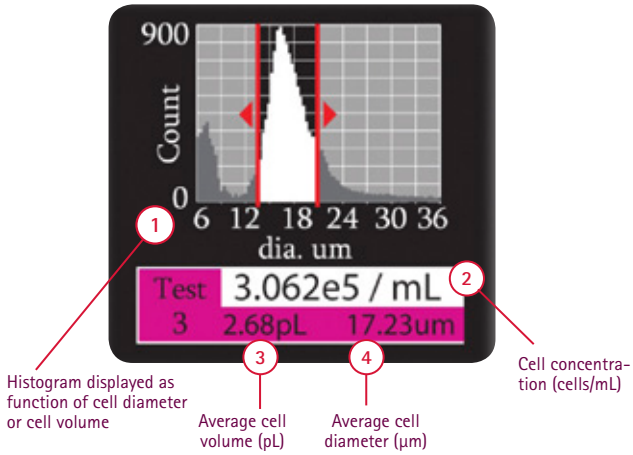
Merck Millipore Validated

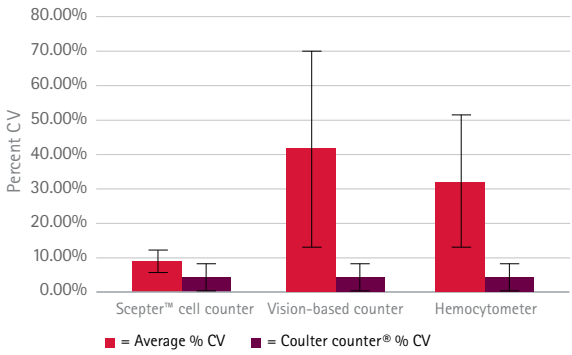
Customer Validated

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

Scepter™ counting delivers precision

There is no need to subjectively determine cell counts, as required by vision-based counting methods. 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.





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

As easy as pipetting

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.

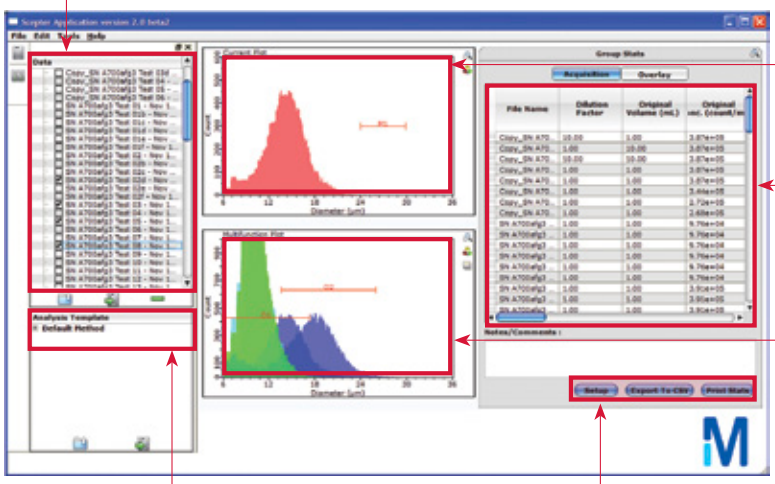
Perform cell count:

- 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 instructions 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.

Intuitive analysis 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

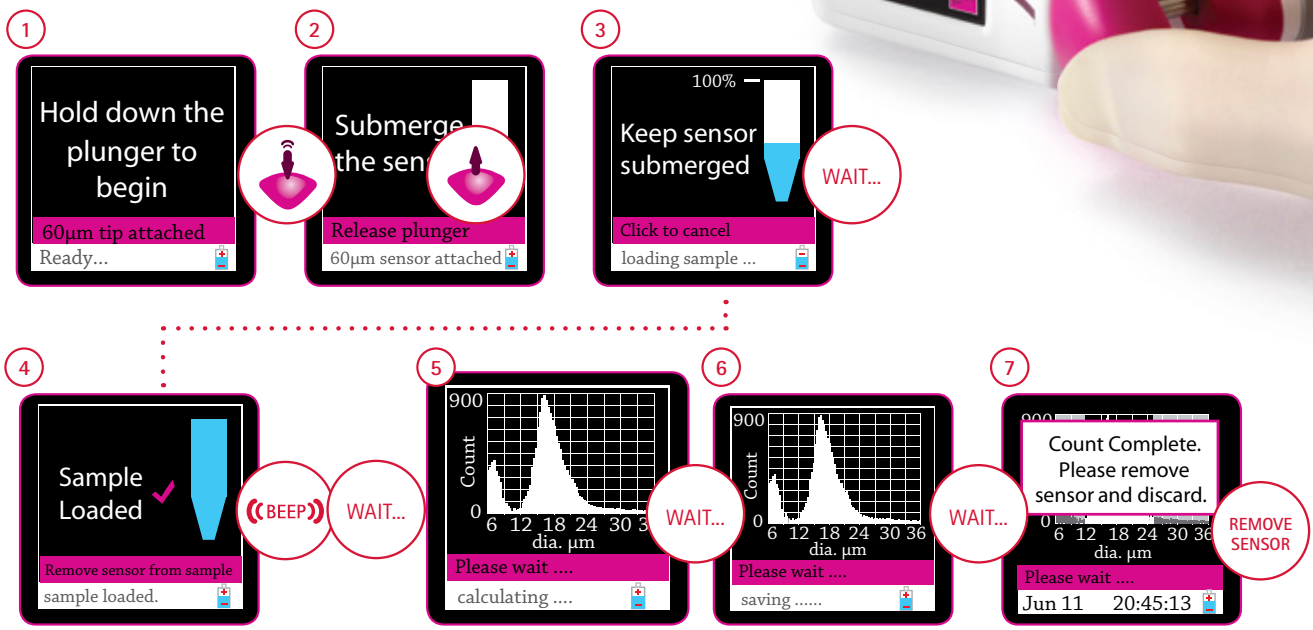
MULTIFUNCTIONAL PLOT: multiple data sets/histogram overlays

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 multiparametric tables
- Create and save gates to be used from one experiment to the next
- Create attractive graphical presentations and reports with your data



Applications Using the Scepter™ 2.0 Cell Counter

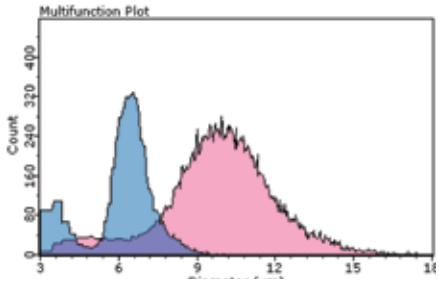
Use the Scepter™ 2.0 Cell Counter to monitor multiple cell types in various applications! We continue to develop new applications for this versatile device; for the most recently developed application notes, please visit: **www.merckmillipore.com/scepter**

Immune Cell Analysis

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.

Download the complete application note at www.merckmillipore.com/scepter

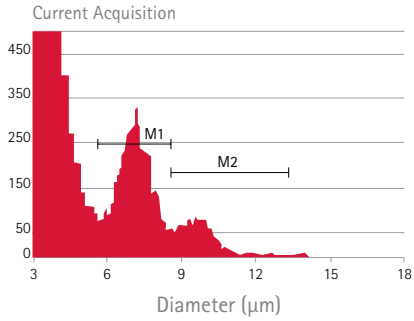


Th1, Th2, and Th17 cells expanded from approximately 6 to 10 µm when compared to the progenitor CD4+ T cell type. This expansion upon differentiation was clearly, accurately, and precisely measured using the Scepter™ cell counter. (Representative Th2 data shown here).

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.

Download the complete application note at www.merckmillipore.com/scepter

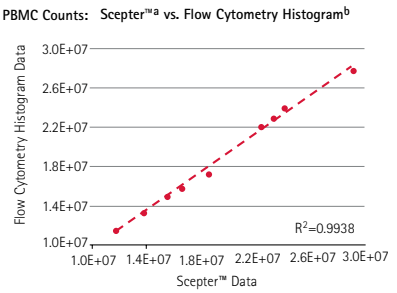


Scepter™ 2.0 counting was used to detect lymphocytes (right hand peak), monocytes (middle peak) and cell debris/dead cells (left hand peak) in human PBMC samples.

Human PBMC Isolation And Counting

This application note presents a rapid, simple, and reliable method of peripheral blood mononuclear cell (PBMC) isolation and subsequent quantitation. We have shown that, by using the new 40 µm aperture sensor, the Scepter™ 2.0 cell counter accurately and precisely counts a much broader range of cell types, including small cells (< 6 µm in diameter) such as PBMC and red blood cells (RBC).

Download the complete application note at www.merckmillipore.com/scepter



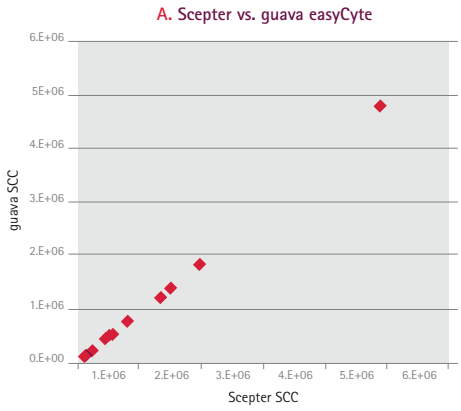
Scepter™ 2.0 cell counter provided PBMC counts that were well-correlated with flow cytometric analysis of stained cells.

Specialized Applications

Rapid Counting Of Somatic Cells In Dairy Milk

Mastitis is an inflammatory disease of the mammary glands in cows characterized by pathological changes in mammary tissue. Milk from sick cows exhibits increased somatic cell counts (SCC) due to the release of white blood cells into the gland to combat infection. Milk with high a SCC is of lower economic value than milk with low a SCC so it is crucial that SCC in monitored. This application note details the sample preparation required to get accurate counts using the Scepter™ 2.0 Cell Counter and correlates that data to flow cytometry results.

Download the complete application note at www.merckmillipore.com/scepter

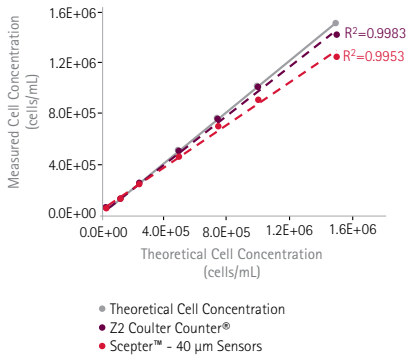


Easy counting of somatic cells in dairy milk. Somatic cells were purified from dairy milk using a simple and reliable spin wash protocol. Despite requiring less sample preparation than flow cytometry, the Scepter™ Cell Counter data correlate well with data from the guava easyCyte™ flow cytometer.

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.

Download the complete application note at www.merckmillipore.com/scepter

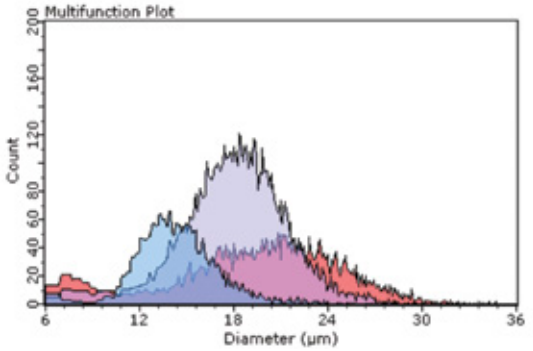


Comparing yeast cell counts from the Scepter™ cell counter and Z2 Coulter Counter®. Measured yeast cell concentrations are plotted against the theoretical concentrations. The solid gray line represents the theoretical values.

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.

Download the complete application note at www.merckmillipore.com/scepter



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

Instrument and Accessories	Catalogue No.
Muse® Cell Analyzer	0500-3115
Muse® Replacement Flow Cell	0500-3120
Instrument Cleaning Fluid (100 mLs)	4200-0140
Muse® System Check Kit	MCH100101
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
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

www.merckmillipore.com/muse
www.merckmillipore.com/scepter

Description	Qty	Catalogue 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	1	
Downloadable Scepter™ Software	1	
O-Rings	2	
Scepter™ Test Beads	1	PHCCBEADS
Scepter™ USB Cable	1	PHCCCABLE
Scepter™ Sensors, 60 µm	50 500	PHCC60050 PHCC60500
Scepter™ Sensors, 40 µm	50 500	PHCC40050 PHCC40500
Universal Power Adapter	1	PHCCPOWER
Scepter™ O-Ring Kit, includes 2 O-rings and 1 filter cover	1	PHCC0CLIP

To Place an Order or Receive Technical Assistance

In Europe, please call Customer Service:

France: 0825 045 645
Germany: 01805 045 645
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645

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