THE MUSE® CELL COUNT & VIABILITY ASSAY PROVIDES IMPROVED ACCURACY AND PRECISION FOR CELL COUNTING AND VIABILITY MEASUREMENTS ACROSS MULTIPLE CELL LINES

Cytek Biosciences

Cytek® Guava® Muse®

CYTEK®
TRANSCEND THE CONVENTIONAL

The Muse® Cell Count & Viability Assay Provides Improved Accuracy and Precision for Cell Counting and Viability Measurements Across Multiple Cell Lines

Introduction

The assessment of cell concentration and viability is important for characterizing cell health. This information can be useful for monitoring proliferation rates, optimizing growth conditions, and normalizing cell data for further studies, such as assessing the effects of cytotoxic compounds. Current methods to generate this data utilize multiple—sometimes complex—instrument platforms, or simpler techniques which rely on single-uptake dyes that can't differentiate between the various states of cellular degradation.

The Cytek® Guava® Muse® cell analyzer was developed to provide comprehensive cell health information rapidly and reliably. It is a robust instrument that enables multi-dimensional cell health analysis using a single platform. It's equipped with a highly-simplified, intuitive touchscreen interface that guides users through acquisition and analysis, and provides rapid measurements for cell concentration and viability, cellular health, cell signaling status, and immunological cell identification using straightforward, mix-and-read assays. Its compact size and ease of use enable researchers with varying experience levels from all over the world to obtain a comprehensive picture of cellular health.

Figure 1. Multidimensional cell health assessment using a single platform.

- Muse® Count & Viability Assay
- Muse® Annexin V & Dead Cell Assay
- Muse® Cell Cycle Assay

The Muse cell analyzer employs multiparametric, fluorescent detection to characterize individual cells via microcapillary flow technology, providing a highly-sensitive and rapid assessment of cellular samples using minimal cell numbers.

The Muse® Count & Viability Assay is a simple, sensitive, and rapid assay that provides cell concentration and viability information (Figure 2). In this application note, we demonstrate that this assay provides improved performance compared to conventional cell count and viability measurements that use Trypan blue exclusion.

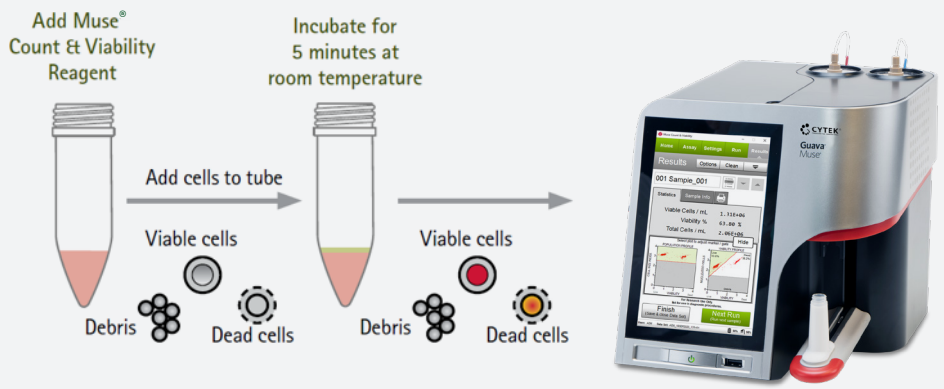
Key features of the assay include:

1. Accurate and precise data provides reliable results
2. Portable instrument with a small footprint saves limited bench space
3. Mix-and-read protocols, rapid measurements, and instantaneous results simplify complex workflows
4. A proprietary combination of two fluorescent dyes enables discrimination between viable and dead nucleated cells
5. Small sample sizes allow users to save precious samples
6. Validation with a range of cellular types, including both suspension and adherent cells, increases confidence in accuracy

The Muse Count & Viability Assay utilizes a proprietary mix of two DNA intercalating fluorescent dyes in a single reagent (Figure 2). One of these dyes is membrane-permeant, and will stain all cells with a nucleus; the other selectively stains cells whose membranes have been compromised and are dying or dead. This combination allows for the differentiation of live cells from dead or dying cells, resulting in accurate and reliable cell concentration and viability results. Stained samples are then analyzed on the Muse® cell analyzer using a guided touchscreen user interface. The Muse Count & Viability Assay displays the results on an easy-to-read results page with an optional plot display. 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.

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Figure 2. Workflow and principle for the Muse® Count & Viability Assay.



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. The assay utilizes a proprietary mix of two fluorescent DNA intercalating dyes to provide information on the 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 will stain with both dyes, but dying cells have lower fluorescence intensity than dead cells.

Materials and Methods

The Muse® Count & Viability Assay uses a highly simplified workflow to provide cell count and viability results, as shown in Figure 2. Sample preparation is very simple with a one-step addition of the mix-and-read Muse® Count & Viability Reagent. Data from prepared samples is quickly acquired using the count & viability software module.

The touchscreen interface workflow for the assay is shown in Figure 3. Briefly, a user enters the count & viability module and hits "Run Assay." The touchscreen prompts the user to load a sample and, through simple on-screen instructions, guides the user through the optimization and verification of settings. The user then enters sample-specific information and selects "Run Sample." The instrument displays the results screen with the calculated concentration values, and provides the user with the option to view dot plots or adjust markers between samples (Figure 3, images).

Data can be stored on the device and exported in a report format or a Microsoft Excel® file, enabling the production of a robust documentation trail with critical experimental details preserved.

Result parameters include information on:

- Number of viable cells per mL
- Percent viability
- Total cells per mL
- Total viable cells
- Total cells
- Dilution factor (input value)
- Original volume (input value)
- Sample number
- Sample ID

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Figure 3. The Muse® Count & Viability Module uses a guided user interface to perform acquisition and analysis in a few quick steps.

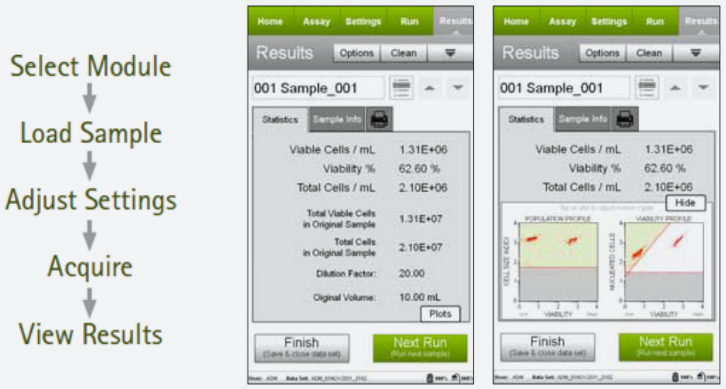


Figure 3. Concentration and viability results are displayed automatically once acquisition is completed. Optional dot plots allow for visualization and further data analysis.

Figure 4. The Muse® Cell Analyzer provides accurate counting of reference counting beads.

Results

Counting Accuracy

The counting accuracy and linearity of the Muse cell analyzer was verified by measuring its ability to provide counts on multiple dilutions of reference counting beads. Figure 4 compares expected bead concentrations to measured bead concentrations using the Muse cell analyzer at multiple concentrations between 1.0 x 10^4 and 1.0 x 10^6 beads/mL. The slopes and correlation coefficients of the linear regression fit curves were both close to 1, demonstrating that excellent counting accuracy and linearity can be obtained using the Muse cell analyzer for the concentration range tested here.

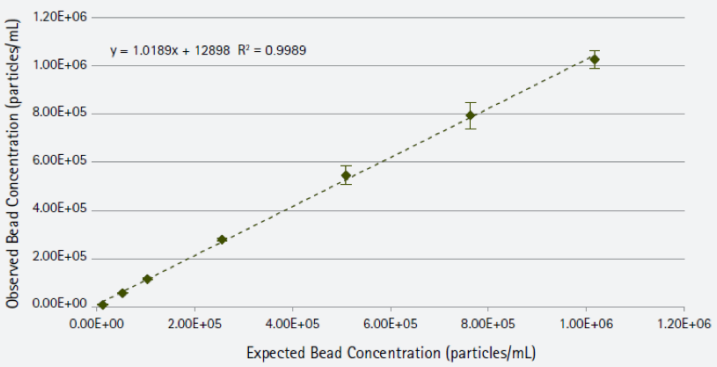


Figure 4. A standard bead solution whose initial concentration was determined using a Coulter Counter® was obtained from an external vendor and diluted over a range of 1.0 x 10^4 to 1.0 x 10^6 particles/mL. Expected bead concentrations were compared to observed bead concentrations. Each point represents the average of triplicate samplings, and error bars represent corresponding standard deviations.

Versatility: Application to a variety of cell lines

The Muse Count & Viability Assay was used to determine cell concentrations across several cell lines, including both suspension and adherent lines. Figure 5 shows the comparison of observed vs. expected cell concentrations for five of the cell lines tested. The theoretical concentrations were calculated based on the serial dilution of the original cell sample, whose concentration was established using the Muse cell analyzer. The slopes and R^2 values for all the cell lines tested closely approached 1, demonstrating that the assay can provide linear responses across a wide range of cell concentrations, as well as diverse cell types.

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Table 1 summarizes the list of cell lines validated to date for use with the Muse Count & Viability Assay. Data from both suspension and adherent cell lines demonstrated accurate count and viability information over a range of sample concentrations.

Table 1. Summary of cell lines tested with the Guava® Muse® Cell Analyzer.

Cell Line	Adherent /Suspension	Origin	Source
Jurkat	Suspension	Acute T Cell Leukemia - Human	ATCC TIB-152
HL-60	Suspension	Promyelocytic Leukemia - Human	ATCC CCL-240
HB-8307	Suspension	B Cell Myeloma - Human	ATCC HB-8307
CHO	Adherent	Ovarian - Chinese Hamster	ATCC CCL-61
SF9	Suspension	Insect Ovary Spodoptera frugiperda	Invitrogen 11496-015
K562	Suspension	Bone Marrow Chronic Myelogenous Leukemia - Human	ATCC CCL-243
MCF-7	Adherent	Breast Adenocarcinoma - Human	ATCC HTB-22
HeLa	Adherent	Cervical Adenocarcinoma - Human	ATCC CCL-2
PC-3	Adherent	Prostate Adenocarcinoma - Human	ATCC CRL-1435

The cell lines tested represent commonly used lines in research laboratories. They include adherent cells, suspension cells, mammalian cell lines, and an insect cell line.

Figure 5. The Guava® Muse® Cell Analyzer performs with high linearity across multiple cell lines and a wide concentration range.

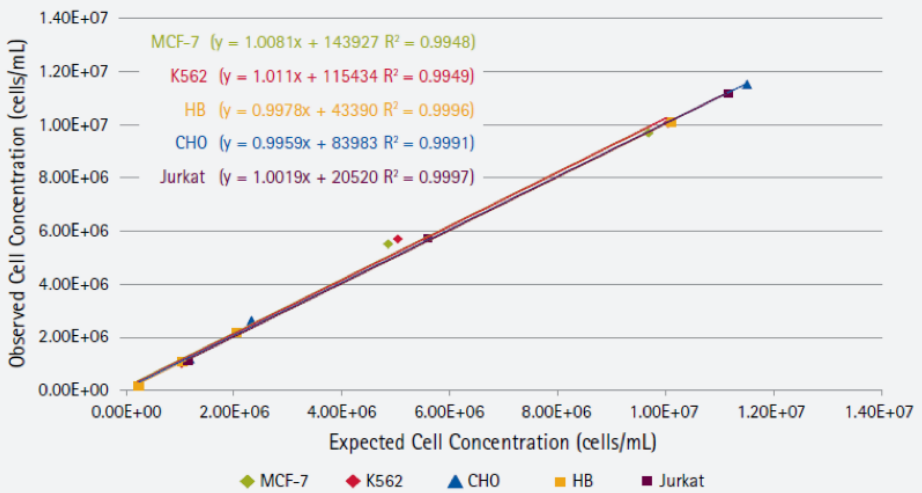


Figure 5. A comparison of observed vs. expected cell concentration results for serial dilutions of five representative cell lines, including both adherent and suspension cells. Each point represents the average of three samplings.

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. Table 2 summarizes the features of each of three methods for cell concentration and viability determination.

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Table 2. Comparison of the Guava® Muse® Cell Analyzer to other devices for measuring cell counts and viability.

	Guava® Muse® Cell Analyzer	Manual Hemocytometer	Automated Imaging-Based Counting Device
Acquisition Sample Format	Tube-based	Slide-based	Slide-based
Staining Method	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 min	Slow due to manual counting	~1 min
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, allowing for reanalysis and reports; Excel® file export option	Lost after being read; manually written down results	Exportable to .csv file-only counts exported

Five different cell lines at multiple concentrations and viabilities were analyzed using the Muse Count & Viability protocol and the manufacturer-recommended protocols for each of the other methods. Figure 6 depicts the comparison of the average of triplicate measurements for each individual cell counting method versus the average cell concentration (calculated by taking the mean average cell concentration from all three methods together).

Regression statistics show that the Muse cell analyzer demonstrates excellent agreement and provides accurate and comparable results to a variety of viability methods and instruments.

Figure 6. The Guava® Muse® Cell Analyzer provides accurate cell concentration measurements, comparable to results from other analysis methods, for both suspension (A) and adherent (B) cell lines.

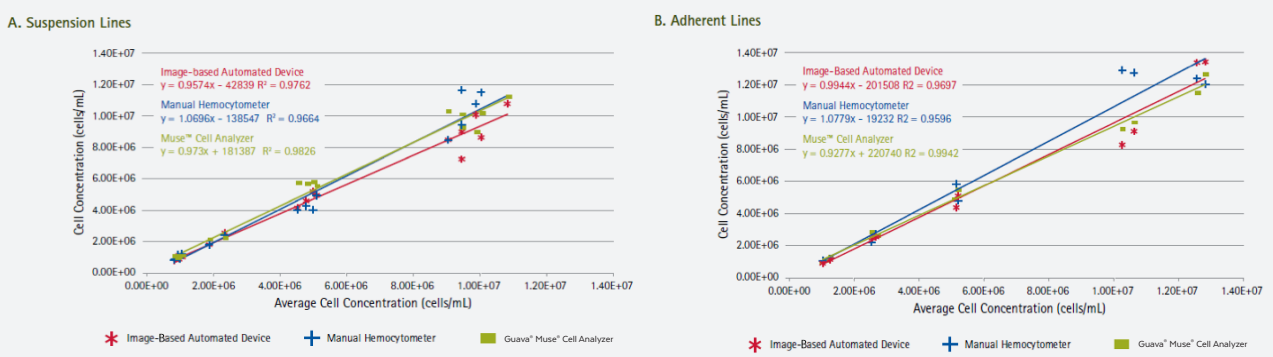


Figure 6. The plots compare Muse® cell concentration data with the combined average of measured cell concentrations from all three counting methods (the image-based automated device, manual hemocytometer, and the Muse cell analyzer). Each point represents the average of triplicate measurements.

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Precision and Reproducibility

The precision of the Muse Count & Viability Assay was evaluated using the analysis methods and studies described above (Figures 5 and 6). Table 3 summarizes the average percent coefficient of variation (%CV). The %CV range was obtained using the three counting methods to analyze 90 cellular samples from both suspension and adherent cell lines at multiple concentrations.

Table 3 demonstrates that the Muse cell analyzer provided an average %CV of 4.0% for cellular concentration determination, which was lower than what was observed for image-based automated counting (9.2%), as well as what was observed for manual hemocytometry (6.3%). 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 a %CV of less than 10% over the entire range of samples tested. Higher %CVs were observed for Trypan blue-based methods, particularly at lower cell concentrations.

Table 3 also shows that the Muse cell analyzer has a lower average %CV (2.2%) for viability measurements compared to the alternative methods. Further, the %CV for viability measurements on the Muse cell analyzer was <7% for all samples tested. The data demonstrates that the Muse cell analyzer can provide superior precision for cell counting measurements using multiple cell lines across multiple concentrations.

Table 3. The Muse® Cell Analyzer provides superior precision for cell concentration and viability measurements compared to Trypan blue-based analyses.

Analysis Method	Cell Concentration		Viability	
	Average %CV	%CV Range	Average %CV	%CV Range
Guava® 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.–15.3%	4.5%	0.5–9.2%

Data was generated from triplicate measurements of 30 cellular samples from suspension and adherent cell lines at multiple concentrations and viabilities.

Figure 7 demonstrates viability results from multiple cell lines at multiple cell concentrations. Low variation between cell viability was seen at each concentration, as shown by the standard deviation bars. The data supports that the Muse cell analyzer provides reliable viability results across a wide concentration range, covering most cell concentrations encountered during standard culturing and cellular research.

Figure 7. Consistent viability results across various cell concentrations and cell types.

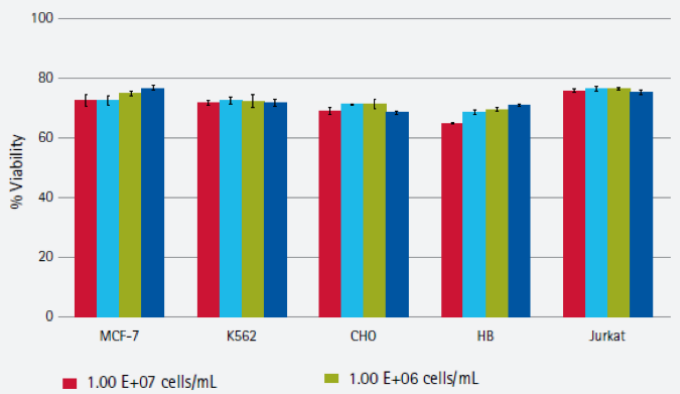


Figure 7. Five cell types from both adherent and suspension lines were harvested and viabilities were determined across various cellular concentrations. Each bar represents the average of triplicate samples, and error bars represent the corresponding standard deviations.

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Conclusions

The Muse® cell analyzer is a robust instrument that enables the measurement of multiple cell health-related parameters on a single platform. Specific assay modules facilitate the rapid, easy assessment of cell health using the Muse assays for counting and viability, apoptosis detection, and cell cycle distribution.

Performance data demonstrates excellent correlations with traditional, accepted analysis methods, and confirms that this platform yields accurate results for a variety of cell types and concentrations. Further, the Muse platform offers superior precision compared to traditional methods for cell counting and viability measurements. By making cell health analysis simple, affordable, and easily accessible, the Muse cell analyzer can help integrate cell health analysis into everyday cell culture workflows. As a result, data from cell-based experiments can be made more consistent and reproducible, enabling faster, more accurate decisions, and more productive research.

For Research Use Only. Not for use in diagnostic procedures.

©2023 Cytek Biosciences, Inc. All rights reserved. Cytek, Guava and Muse are trademarks of Cytek Biosciences, Inc. All other trademarks are the property of their respective owners.

CYTEK®
TRANSCEND THE CONVENTIONAL

FL213046
April 2023

Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com