Application Forum

Precise and Accurate Counts and Viability Measurements Across Multiple Cell Lines Using the Muse™ Cell Count & Viability Assay

EMD Millipore

Introduction

The assessment of cell concentration in combination with viability is an important step in the characterization of cell health. Cell concentration and viability information can be used for monitoring proliferation rates, optimizing growth conditions and normalizing cell data for further studies, such as assessing the impacts of cytotoxic compounds. Current methods that rely on multiple instrument platforms to provide these answers reduce flexibility, limit the ability to obtain comprehensive cell health information and add increased costs to researchers. Other, simpler methods provide inconsistent results due to their dependence upon single-uptake dyes, which do not effectively discriminate between the various states of cellular demise. Therefore, there is a crucial need for analytical methods that provide rapid, robust and reproducible count and viability data to enable the efficient, daily execution of cellular research.

The Muse™ Cell Analyzer is a unique instrument that enables multidimensional cell health analysis on a single platform. The simplified format enables researchers of varying backgrounds and experience levels to obtain a comprehensive picture of cellular health. This small, robust benchtop cell analyzer effortlessly guides users through the acquisition and analysis of samples using mix-and-read assays with a highly simplified and intuitive touchscreen interface which delivers rapid measurements of cell concentration, viability, apoptotic status, and cell cycle distribution. Using multiparametric fluorescent detection of individual cells via microcapillary flow technology, the system enables highly sensitive and rapid detection of cellular samples using minimal cell numbers.

The Muse™ Count & Viability Assay is a simple, rapid, linear assay for cell concentration and viability (Figure 1). We show that the assay can outperform viability and count measurements that use Trypan blue exclusion.

The assay utilizes a proprietary mix of two DNA intercalating fluorescent dyes in a single reagent (Figure 1). One of the dyes is membrane permeant and will stain all cells with a nucleus. The second dye only stains cells whose membranes have been compromised and are dying or dead. This combi-

nation allows for the discrimination of nucleated cells from those without a nucleus or debris, and live cells from dead or dying resulting in both accurate cell concentration and viability results. Stained samples are then analyzed on the Muse™ Cell Analyzer using a guided touchscreen user interface. The Count & Viability Assay display results in 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.

Materials and methods

The Muse™ Count & Viability Assay uses a highly simplified workflow, as shown in Figure 1. Sample preparation is very simple with the one-step addition of a mix-and-read reagent. Data from prepared samples are quickly acquired using the touchscreen Count & Viability Software Module.

Briefly, a user enters the 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

Figure 1. 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.

Figure 2. The Muse™ Cell Analyzer performs with high linearity across multiple cell lines and a wide sample concentration range. 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. Other cell lines tested included HL-60 promyelocytic leukemia cells (suspension), PC-3 prostate adenocarcinoma cells (adherent), HeLa cells (adherent), and SF9 insect cells (suspension). Each point represents the average of three samplings. "HB" = HB-8307 human myeloma cells.

user then enters sample-specific information and then touches "Run Sample." The instrument displays the calculated concentration values and provides the option to view the dotplot as well as adjust markers between samples.

Results and discussions

The Muse™ Count & Viability Assay was used to determine cell concentration across several cell lines, including both suspension and adherent lines, at a variety of concentrations. Figure 2 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 R² values for all the cell lines tested closely approached 1, demonstrating that the assay can provide accurate and linear responses across a wide range of cell concentrations as well as diverse cell types.

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:

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

Five different cell lines at multiple concentrations and viabilities were analyzed using the Muse™ Count & Viability protocol and manufacturer-recommended protocols for each of the other methods. Figure 3 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.

Precision and Reproducibility

The precision of the Muse™ Count & Viability Assay was evaluated using the analysis methods and studies described above (Figures 2–3). Table 1 summarizes the average percent coefficient of variation (%CV) and %CV range obtained using the three methods to analyze 90 cellular samples from suspension and adherent cell lines.

The table demonstrates that the Muse™ Cell Analyzer provided average %CV of 4.0% for cellular concentration determination, which was lower than that observed for image-based automated counting (average %CV of 9.2%) and lower than that observed for manual hemocytometry (average %CV of 6.3%). The Muse™ Cell Analyzer exhibited narrower range of %CVs than other methods 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.

The Muse™ Cell Analyzer has a lower average %CV (2.2%) for viability measurements compared to the other methods. The %CV for viability measurements on the Muse™ Cell Analyzer was < 7% for all samples tested (Table 1).

Figure 4 demonstrates viability results from multiple cell lines at multiple cell concentrations. Low variation between

A. Suspension Lines

B. Adherent Lines

Figure 3. The Muse™ Cell Analyzer provides accurate cell concentration measurements, comparable to results from other analysis methods, for both suspension (A) and adherent (B) cell lines. The plot represents comparison of Muse™ cell concentration data with combined average measured cell concentrations from all three counting methods (Image-Based Automated Device, Manual Hemocytometer and Muse™ Cell Analyzer). Each point represents the average of triplicate measurements.

Table 1. 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
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%

Data are based on triplicate measurements of 30 cellular samples from suspension and adherent cell lines at multiple concentrations and viabilities.

Figure 4. Consistent viability results across various cell concentrations and cell types. Five cell types representing both adherent and suspension lines were harvested and viabilities determined across various cellular concentrations. Each bar represents the average of triplicate samplings and error bars represent their corresponding standard deviations.

viabilities at each concentration was seen, as shown by the small standard deviation bars. The data support 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.

Conclusions

The Muse™ Cell Analyzer is a multifaceted instrument that enables measurement of multiple cell health-related parameters on a single platform. Specific assay modules facilitate rapid, easy assessment of cell health using assays for counting and viability (shown in the present study), apoptosis detection and cell cycle distribution.

Performance data demonstrate excellent correlations with traditional, accepted analysis methods and confirm that

this new platform yields accurate results for a variety of cell types and concentrations. Furthermore, the Muse™ platform yields superior precision compared to traditional methods of cell counting and viability measurement. 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, cell-based experiments can be made more consistent and reproducible, enabling faster, more accurate decisions for more productive research.

Sponsored Paper. *BioTechniques* 52:200-203 (March 2012)
doi 10.2144/000113827

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