

A Better Alternative to Traditional Methods for Cell Counting and Viability Assessments?

New Platform for a Broad Range of Cell-Based Assays

By Glenn Terashita, Guava Technologies Inc



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Measurement of cell counts and viability is an essential task done routinely in any biology lab working with cells. Yet, a decades-old methodology is in near-universal use even today for generating this basic information.

Tedium Defined

From an aspiring biologist’s first laboratory course in college, the hemacytometer with its embedded grid pattern becomes a basic tool of his or her trade. Biologists spend countless hours over the course of their training and careers staring at these grids through a microscope. Were this not enough of a validation of one’s dedication to their field, the trypan-blue dye exclusion chemistry routinely used for cell counting requires mastering the scientific art of interpreting and classifying particles based on their size and “blueness” characteristics.

Limitations of Trypan Blue

The interpretation of these multiple shades of blue or blue-gray results in subjectivity and imprecision, particularly when technician-to-technician factors are considered. In addition, trypan blue chemistry has been reported in the literature to consistently overestimate cell viability.^{1,2}

A New Alternative

A company by the name of Guava® Technologies has quietly developed a significant following over the past year and a half with the commercial availability of the automated Guava ViaCount® assay for cell

	Cells/mL	% of Total	Cells in Original Sample
Viable	1.06e06	84.70%	1.06e07
Apoptotic	5.92e04	4.75%	5.92e05
Dead	1.32e05	10.55%	1.32e06
Total	1.25e06	100.0 %	1.25e07
<i>Dilution Factor</i>	20	<i>Original volume</i>	10 mL

Unique to the Guava ViaCount assay is an analysis option to include assessment of the apoptotic fraction.

counting and viability assessment. (This is but one of several assays available from Guava; we will touch on these later in this article.)

The ViaCount assay is run on the Guava PCA™ instrument, an automated platform for cell-based assays. ViaCount chemistry is based on a combination of two nucleic acid dyes used to identify cells while excluding cellular debris, and to distinguish live cells from dead. This approach also offers the unique ability to simultaneously assess the apoptotic cell fraction within a sample.

Also of note is the fact that the ViaCount assay protocol calls for only 20µL of the original cell sample, conserving often-precious cells. This contrasts with the 1mL of sample consumed by other automated cell counting systems, 50 times the volume used by the Guava system.

A Robust Assay

The Guava ViaCount system consists of the Guava PCA instrument, a laptop computer with the ViaCount software module, and the ViaCount reagent.

	Not Counted	Viable Cell	Non-viable Cell
Nuclear Stain (PM2)	—	+	+
Forward Light Scatter	LOW	HIGH	HIGH
Viability Stain (PM1)	—	—	+

The ViaCount assay uses 3 correlated criteria to assess cell counts and viability.

The system provides a total cell count, viable cell count and percentage, and optionally, an apoptotic cell count and percentage. The system does not require an additional reagent to assess the apoptotic cells.

The Guava PCA instrument is based on a unique capillary flow cell technology, enabling rapid analysis of individual cells. The detection system is laser-based for high sensitivity.

The ViaCount assay bases its results on a 3-parameter correlated assessment of each “event” detected by the system. The size of each particle passing through the flow cell is measured along with its fluorescence in two spectrally distinct channels or wavelengths.

The ViaCount reagent includes two DNA intercalating dyes. The first of these dyes is membrane permeant, entering and staining all nucleated cells. The second dye is a viability dye which penetrates and stains dead and dying cells with compromised membrane integrity, and is excluded by cells with intact membranes.

Guava has shown that cells which take up intermediate amounts of the viability dye also react with Annexin V, indicating they have entered the apoptotic pathway.

In comparison with trypan blue-based methods, the Guava ViaCount assay provides a more accurate and complete evaluation of cell cultures.

Flexible Assay Platform

Intriguing as the Guava ViaCount assay may be, it is just one of several assays available for the Guava PCA platform. Each of the Guava assays includes a dedicated software module, which contributes to overall ease-of-use of the system for multiple assays.

For more detailed assessment of apoptosis across a broad range of applications, Guava offers apoptosis assays for Annexin V, caspases, and TUNEL.

Also available is the Guava Express assay for flexible analysis of cell surface and intracellular staining. Examples of applications include evaluation of transfected cell lines, screening of hybridomas, detection of cell surface protein expression, and many others.

The latest addition to the Guava PCA menu is the cell cycle assay for assessment of cell cycle phases.

Most of these additional assays are typically performed on a flow cytometer, an expensive and complex technology usually requiring a specially trained, dedicated operator. If you have access to a flow cytometry facility, it is probably through a core lab, requiring scheduling of time well in advance of your experiment.

Another key advantage of the Guava PCA system is the capillary flow cell. Because of this innovative technology, assays require only a small number of cells per test, conservatively on the order of one-tenth the number required to run equivalent assays by flow cytometry.

In summary, the Guava ViaCount assay is worth considering solely on its merits as an improved and more comprehensive alternative to other methods for cell counting and viability assessments. In addition, a menu of sophisticated cell-based assays is available on the Guava PCA platform, a simple, compact, affordable system which provides access to capabilities previously out of reach for most laboratories.

Footnotes

- 1 Altman SA, et al. *Biotechnology Progress* 9: 671-674, 1993
- 2 Mascotti K, et al. *Transfusion* 40: 693-696, 2000

Product Notes

Products sold by Guava Technologies are intended for in vitro research use only. A technically qualified individual should supervise usage. Individuals receiving this information must exercise independent judgments in determining its appropriateness for a particular purpose. Material Safety Data Sheets are available for each product. Contact Guava Technologies Technical Support for the most up-to-date information.

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