Understanding the Relationship between Automation/Instrumentation and Microplates

SnAPPShots

A brief technical report from the Corning Applications Group

Katherine E. Strathearn Ph.D. and Mark Rothenberg Ph.D.
Corning Incorporated, Life Sciences
Kennebunk, Maine

Introduction

The past 30 years have seen an astonishing rate of growth in automation to aid in research and drug development. From instruments such as basic microplate readers, liquid handlers, grippers, and incubators, to highly advanced instrumentation such as high content imagers, the microplate-automation relationship has always played a critically important role.

The relationship between microplates and the equipment used to run the experiment is fundamental to any assay ranging from low to high throughput. By not appreciating this dynamic, resulting data will be less robust, CVs will be increased and there will be more opportunities for false results. For instance, if a researcher wants to perform a fluorescence-based assay, selecting a clear microplate would result in not only high background but also crosstalk between wells resulting in inferior data. Additionally, if the microplate reader is optimized for a 384 well normal volume microplate and a researcher attempts to analyze a 384 well low volume microplate, the microplate dimensions and focal plane will be inaccurate leading to suboptimal results. Factors such as those listed above and others (e.g., liquid handling) are critical to understand when optimizing any assay.

The following describes the importance of choosing the correct microplate for an assay, as well as optimizing the reader for the microplate. Additionally, the importance of proper liquid handling and how inaccurate dispensing of a sample may negatively impact the results is presented. The goal is to arm researchers with the tools needed to develop successful assays by providing a better understanding of the relationship between microplates, assays, and instrumentation.

Materials and Methods

- Multitox-Glo was purchased from Promega (Cat. No. G9272), and the protocol was followed as outlined in the manual.
- Fluorescein was purchased from Sigma (Cat. No. F6377)
- The following Corning® microplates were used:
  - 384 well Black Solid Bottom (BSB) Tissue Culture Treated (TCT) microplate (Cat. No. 3571)
  - 384 well White Solid Bottom (WSB) TCT microplate (Cat. No. 3570)
  - 384 well BSB Not Treated (NT) Standard microplate (Cat. No. 3573)
  - 384 well BSB NT Low Volume (LV) microplate (Cat. No. 3821)
- Plates were screened using an EnVision® multimode reader (Perkin Elmer).

Results and Discussion

The Value of Choosing the Correct Microplate

In any assay, selecting the correct microplate is critical for assay success; it is essential to understand that each well contains its own microenvironment for any biochemical or cell-based assay. Microplate dimensions (e.g., well geometry, depth, and surface area), surfaces and color all play important roles in achieving optimal results. Therefore, when designing an assay the following questions should be addressed (Fig. 1):

CORNING

1. What type of assay will be performed? (i.e., What type of surface will be needed?)
   a. Biochemical:
      i. Not treated
      ii. Nonbinding
      iii. Medium binding
      iv. High binding
   b. Cell-Based:
      i. Tissue culture treated (TCT)
      ii. Corning® CellBIND® Surface treated
      iii. Poly D-lysine (PDL) coated
      iv. Ultra-Low Attachment Surface
      v. Biological coated microplates (collagen, laminin, etc.)
2. What type of detection is required?
   a. Fluorescence
      i. Fluorescence intensity
      ii. Time resolved fluorescence-like assays
   b. Luminescence
   c. Absorbance
   d. Label-free (e.g. Epic® technology)
3. What type of reader is required?
   a. Top reader
   b. Bottom reader
   c. Top/bottom reader
   d. High content imager
4. Microplate density and type?
   a. 96 well normal volume or 96 half area microplates
   b. 384 well normal volume or low volume microplates
   c. 1536 well microplates
   d. Polystyrene
   e. Polypropylene cyclic olefin copolymer (COC) – storage and chemical resistance

As mentioned above, selecting the correct microplate for the assay is critical for a successful experiment; it is important to understand when to use a black, white, or clear microplate (Table 1). Typically for luminescence-based assays, due to the low energy produced by the biochemical reaction releasing light, the recommendation is to use a white colored microplate. The white colorant allows for increased reflective capacity for data capture.

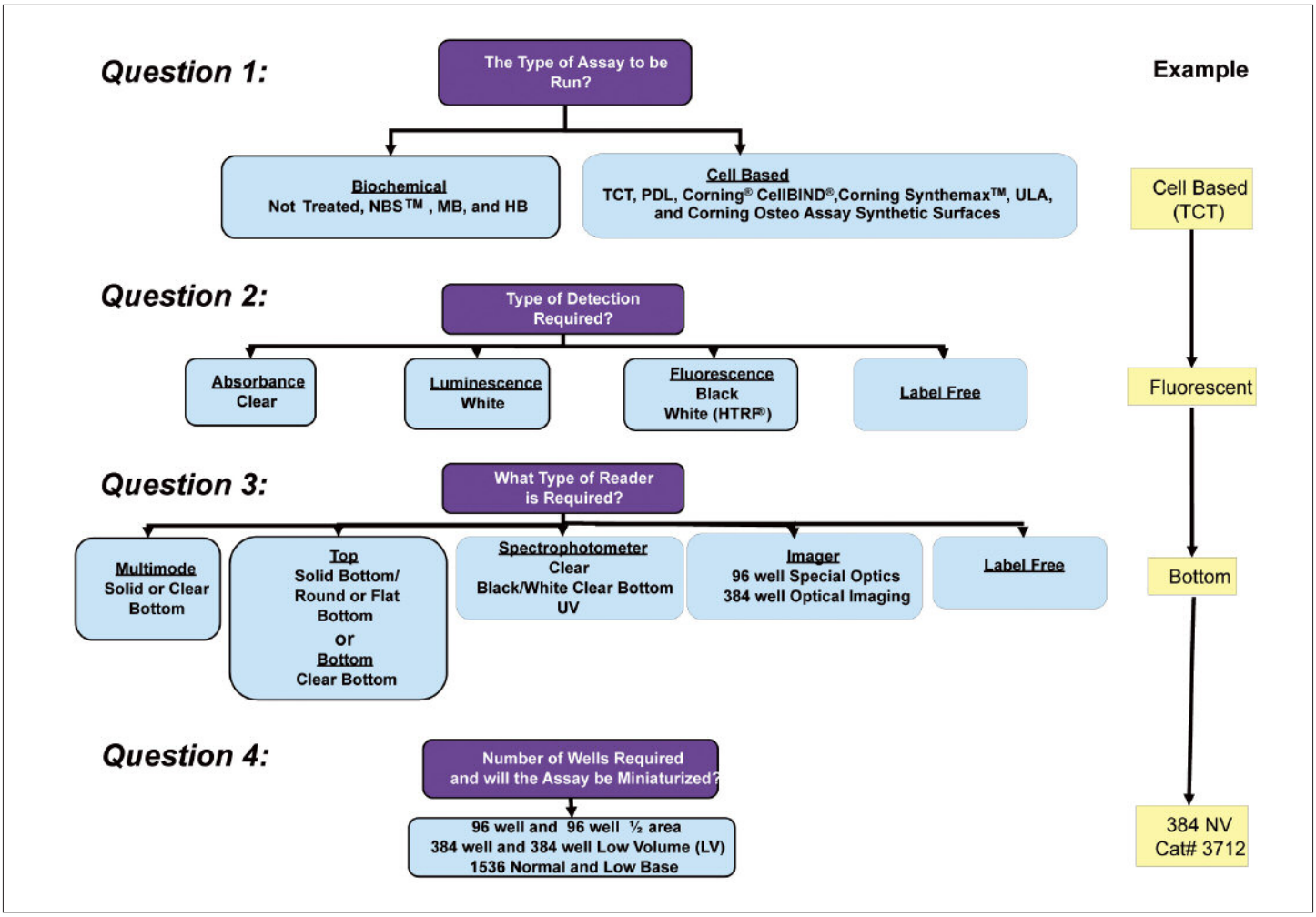


Figure 1. Flow chart designed to select the best microplate for the assay and instrument.

It is widely accepted that for fluorescence intensity assays, a black colored microplate is the optimal choice. Fluorophores, when excited by an energy source, release a large amount of energy in the form of light. The black colorant used in the microplates helps to reduce background by absorbing some of the emission energy as well as preventing cross-talk between wells. Background or noise is of critical concern in any fluorescent assay. Utilization of a clear microplate for a fluorescence-based assay may lead to photobleaching, photochemical destruction of a fluorophore, and high crosstalk levels between wells resulting in a low signal to noise ratio. For any assay, a high signal to noise ratio is preferred to reduce the possibility of false-positives and negatives. A low signal to noise ratio may mask subtle differences in the fluorescent signal leading to the potential of false negatives.

In most cases, a white microplate for a fluorescence-based assay may also lead to a poor signal to noise ratio because the overall signal (including background) will be amplified. However, this result is dependent on whether the fluorescent dye is a high or low energy emitter. A low energy emitter yields better results in a white microplate compared to a black microplate. For example, in Time-Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) assays a stable, low energy emitter fluorescent molecule is used to examine protein-protein interactions. As a result, a white microplate would be preferred due to its ability to amplify the low signal produced by the protein interactions. Fig. 2 depicts another instance when selecting a white microplate would be optimal to achieve the highest fluorescent signal.

Understanding the impact of surface area, well depth, and well number is important when choosing a microplate. When performing cell-based assays it is vital to know the surface area of that given microplate. Surface area is a distiguishing feature between vendors who sell microplates. It is highly recommended that when seeding microplates for any cell-based assay that seeding is performed by surface area (cells/cm^2) rather then cells/well. Not all microplates have the same surface area despite having the same number of wells (e.g., normal volume (NV) vs. LV microplates). For detailed information on microplate dimensions and compatibility with various readers please visit http://www.corning.com/lifesciences/us_canada/en/technical_resources/product_guid/eq_cp.aspx. It may be beneficial to consider miniaturizing an assay into a LV microplate so that the same microplate format can be used while reducing reagent consumption, cost, and still minimizing impact on automation.

Lastly, when selecting a microplate for an assay, it is essential to understand how the microplate surface can impact the results. As mentioned above, there are different surface types based on the assay that is being performed (biochemical or cell-based). When performing biochemical assays such as an enzymatic assay, it may be best to use Corning's NBS™ microplate. This microplate is designed to significantly reduce (<2 ng/cm^2) protein and nucleic acid binding thus providing researchers with maximum accessibility of assay components in solution compared to other microplates. However, due to the nature of the coating, the NBS microplate is not recommended for cell-based assays. For biochemical reactions where proteins (antibodies, antigens etc.) are required to bind to the surface, the better option is to use of the High Binding (HB) surface microplate. The HB microplate enhances binding of medium to large biomolecules (>10 kD) that are positively charged, with or without hydrophobic regions. Furthermore, when performing cell-based assays it is necessary to use a microplate that is designed for cell attachment (e.g. TCT, Corning® CellBIND® Surface or PDL). While some cells adhere well under normal conditions, the cells may detach during an assay with multiple wash steps. If cells are detaching during an assay one option would be to switch to a microplate precoated with PDL or

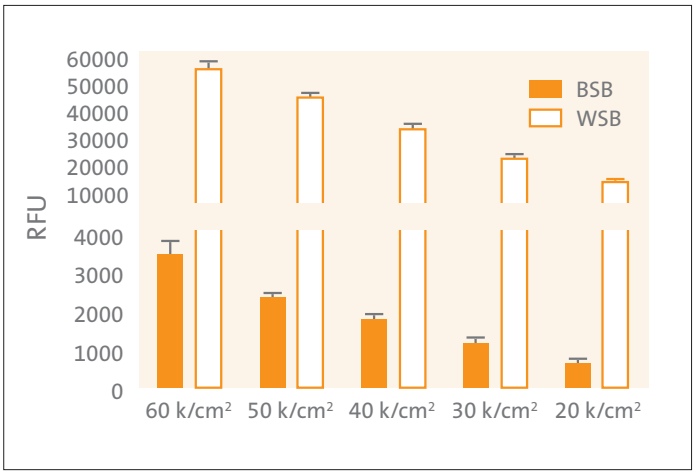


Figure 2. CHO-K1 cells were plated at various seeding densities for 24 h in phenol red- free 2% FBS containing media. Cells were incubated with MultiTox-GLO GF-AFC reagent for 1 h at 37°C. Fluorescent signal was measured with the Perkin Elmer EnVision® instrument λ_{ex} 400 nm, λ_{em} 515 nm). The results demonstrate that a white solid bottom (WSB) microplate yields higher relative fluorescence units (RFU) than a black solid bottom (BSB) microplate.

Table 1. Selecting the Best Microplate for the Assay

Detection Type	Sensitivity	Background	Handling	Plate
Colorimetric	Low	Sometimes	Very easy	Clear
Radiometric	Very high	No	Difficult	White
Luminometric	Very high	No	Easy	White
Fluorometric	Medium to High	Varies	Easy	Black*
Label Free	Very high	No	Easy	Black with optical biosensors

*Note that some assays require a white microplate for optimal results.

Corning® CellBIND® Surface treated. Alternatively, other biological coatings could be used (e.g., collagen and laminin); however, the coatings and concentrations would need to be optimized for each cell line. Figure 3 provides an example on how selecting the correct microplate surface is important for assay optimizations. For more detailed information on recommended microplate surfaces please visit http://www.corning.com/lifesciences/us_canada/en/technical_resources/surfaces.aspx.

Importance of Instrumentation Settings

An essential part when setting up an experiment and validating all the necessary components (reagents, microplate compatibility, instrument settings, etc.), is to confirm the instrument settings as they relate to the microplate. Settings to consider include, but are not limited to, understanding the various attributes of the instrument (bottom reader vs. top reader, etc.), Z-height, and microplate dimensions (e.g., depth of well, well position, length and width of microplate).

Z-height is defined as the height between the well bottom and focal plane. Well bottom elevation and the volume of liquid will impact the optimal Z-height for an experiment (Fig. 4A). Therefore, for each experiment a researcher would want to re-optimize the Z-height if there is a change in either the microplate type (well height and/or well geometry) or liquid volume. Often minor changes in the Z-height can result in a decrease in signal and a potential increase in the overall microplate CV (coefficient of variance, a statistical measurement that analyzes the distribution of data points around the mean) (Fig. 4B). In the example shown in Fig. 4B, the optimal Z-height is 4 mm. By either increasing or decreasing the height, the signal in this example expressed as relative fluorescence units (RFU), and the CV are greatly impacted.

Confirming and validating the correct microplate dimensions in an instrument is important to achieving optimal results. For example, analyzing a 384 well LV microplate under the settings for a 384 well NV microplate may result in less then optimal results due to higher then expected microplate CVs (Fig. 5A). Figure 5B shows the differences in well geometry

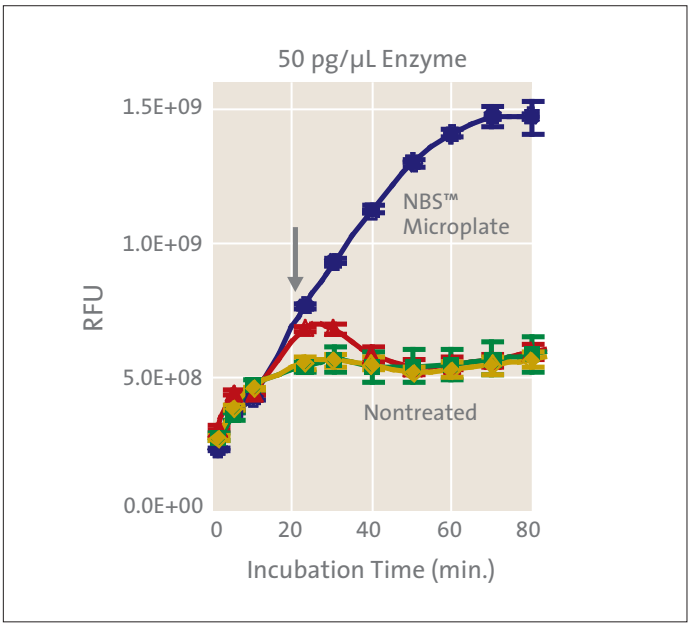


Figure 3. Selecting the correct microplate surface is important for all assays. In the example depicted above two different microplate surfaces were used for an enzymatic assay, NBS™ and Not treated. The data demonstrate that the NBS microplate greatly enhances enzymatic activity compared to the not treated surface. The increase in activity with the NBS microplates is an effect of the lack of protein and nucleic acid binding to the microplate thereby providing researchers with maximum accessibility of the components in solution.

dimensions between the Normal and low volume 384 well microplates. Proper alignment and optimization is critical when changing microplate formats. As depicted in the figure, there is a significant difference between the well depth of the NV microplate (11.43 mm) and the well depth of the LV microplate (9.39 mm). This difference in well depth will greatly impact the optimal Z-height.

Importance of Proper Liquid Handling Techniques

In addition to optimizing Z-height, accurate dispensing of liquids into a microplate is important for obtaining high

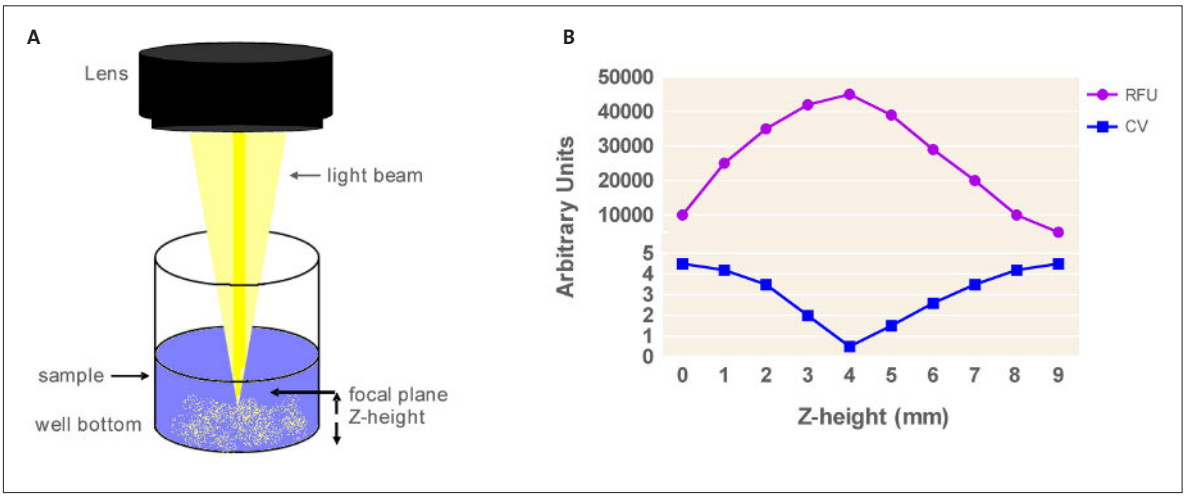
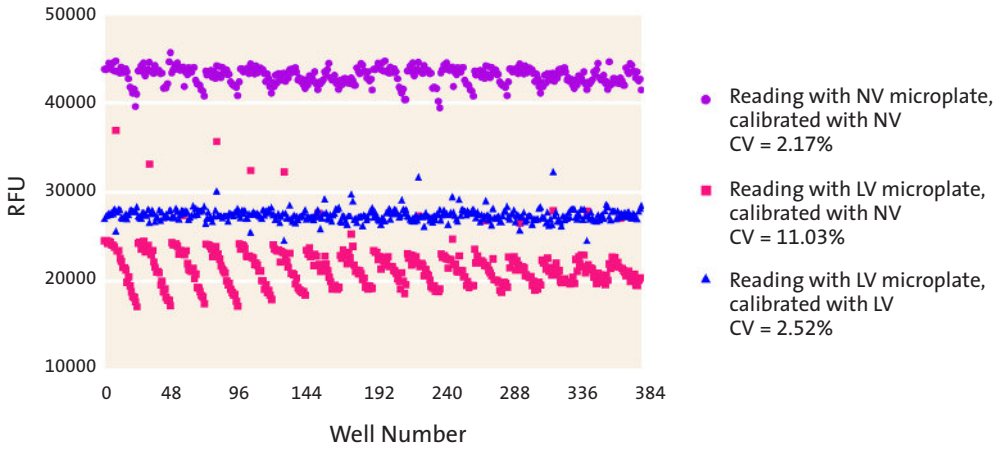


Figure 4. (A) Z-height is the distance between the lens and the sensing volume and determines the focal plane. (B) Performing an assay with suboptimal Z-height can result in a decrease in signal and increase in CV.

quality data (e.g., excellent CVs). If each well in a microplate had a different volume as a result of poor liquid handling, the optimal Z-height for each well would be different. As a result, the data would be inconsistent from well to well leading to high CVs and the possibility of false-positives and negatives. Fig. 5C demonstrates that the same XY coordinates used for a larger well microplate (e.g., 384 NV) may not work for a smaller well microplate (e.g., 384 LV) due to the smaller well diameter. Additionally, for label-free assays accurate liquid handling parameters are essential for optimal results. Taken together, microplate readers, handlers and liquid dispensers should be optimized for each new microplate type, and it is highly recommended that the instrument be re-optimized prior to setting up any new assay.

A



B

	Representative microplate (Corning Cat. #)	Well Volume (uL)	Well Depth (mm)	Well Diameter (top/bottom) (mm)	Plate Length (mm)	Plate Width (mm)	Plate Height (mm)	A1 Row Offset (mm)	A1 Column Offset (mm)	Well center to well center spacing (mm)	Flange or skirt height (mm)	Stack Height	Well Bottom Elevation (mm)	Well Bottom Thickness (mm)	Well Bottom Area (cm2)	Distance to bottom of plate
384 Well Microplates																
Solid black, white, and clear polystyrene plates																
384 low flange flat bottom	3570, 3571, 3572, 3573, 3574, 3575	112	11.43	3.63 / 2.82 (width)	127.8	85.5	14.2	8.99	12.12	4.5	2.41	12.95	2.794	1.4	0.062	1.37
384 low volume flat bottom	3820, 3821, 3822, 3824, 3825, 3826	50	9.39	3.30/2.00	127.8	85.5	12.19	8.99	12.12	4.5	2.41	10.49	2.79	0.635	0.0314	2.165

A1 Row Offset (mm) = Distance from top of plate to center of A1 well; A1 Column Offset (mm) = Distance from left edge of plate to center of A1 well

C

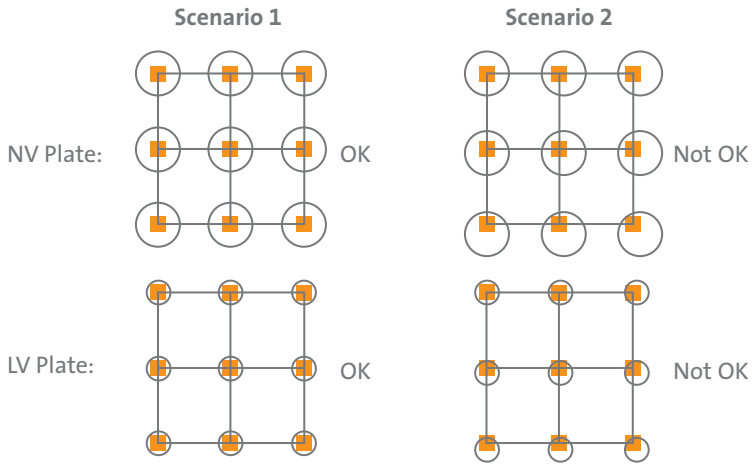


Figure 5. (A) Reading a LV microplate calibrated to a NV microplate results in a more dispersed signal across the microplate and a higher CV value. As demonstrated above a LV microplate calibrated to LV settings (red triangles) results in a uniform signal across the microplate. However, the same microplate calibrated to NV settings (green squares) leads to a high degree of dispersion across the microplate. This effect is due to the differences in the well diameter and depth between the two microplates. (B) Microplate dimensions for a 384 NV and LV microplate. (C) Dispensing liquid with the proper XY coordinates is more important in a LV microplate compared to a NV microplate due to the smaller area of the well. Poor liquid handling may lead to a scattering of data points across a microplate as seen in (A).

Summary

- Each microplate needs to be carefully selected for the assay.
- Instrumentation settings for each assay need to be optimized to the microplate being used.
- Proper liquid handling is important for achieving optimal CVs.
- Collectively, each of these areas needs to be evaluated before a successful assay can be attempted.

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 1.800.492.1110. Outside the United States, please call 978.442.2200 or contact your local Corning sales office listed below.

CORNING

Corning Incorporated
Life Sciences
Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 0402-794-347

China
t 86 21 2215 2888
f 86 21 6215 2988

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

Corning Incorporated, One Riverfront Plaza, Corning, NY 14831–0001