Corning® 384 Well Low Volume Microplate Performance in Miniaturized Assays
Application Report

Innovative Techniques in Drug Discovery

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Summary
In response to the ever-increasing demand for microplates that facilitate assay miniaturization without compromising assay integrity, the new Corning 384 well Low Volume (384 LV) microplates were evaluated for assay performance using a homogeneous, fluorescence polarization protease assay. Following assay miniaturization to 20, 10, 5, and 1 µL working volumes and reagent concentration reduction, the Corning 384 well LV medium bind (MB) and non-binding surface (NBS™) coated assay plates maintained superior signal to noise ratios compared to a competitor’s 384 well small volume assay plate. In addition, when working with very low volumes of 5 and 1 µL/well, Corning 384 well LV NBS coated assay plates had significantly greater signal-to-noise ratio than the competitor’s 384 well assay plate as well as standard 1536 well assay plates. These results suggest that Corning 384 well LV MB and NBS coated assay plates maintain assay integrity while facilitating assay miniaturization to very low reagent concentrations and final working volumes.

Introduction
The trend towards assay miniaturization for high-throughput and ultra-high-throughput screening continues to spur development of homogeneous, fluorescence-based assays in higher density, smaller volume microplate formats (1). Fluorescence techniques are well suited to high-throughput as they provide increased sensitivity, which allows for assay miniaturization. Assay miniaturization reduces the amount of biological and chemical reagents used per assay and better supports the high-throughput screening industry by reducing costs. However, low signal-to-noise ratios resulting from assay miniaturization may result in the loss of potential drug candidates during compound screenings, thus negatively impacting the development and delivery of drugs to market.

Fluorescent techniques, such as fluorescence polarization (FP), are among the most widely used detection approaches for high-throughput screening given the industry-wide drive to simplify, miniaturize, and harness the discovery potential of large numbers of compounds efficiently, both by reducing time and cost, and increasing information content (2). FP assays are highly sensitive and allow one to measure changes in the rotational
In response to the ever-increasing demand for microplates that facilitate assay miniaturization without compromising assay integrity, Corning® now offers its 384 well Low Volume (384 LV) microplates with their unique square to round well geometry. These plates are available with a choice of two surfaces: the standard medium bind (MB) surface and an enhanced non-binding surface (NBS™) that enable superior performance in miniaturized assays. Corning’s NBS technology reduces non-specific binding of molecules to the surface of microwells, thus maintaining them in solution and enhancing assay performance, as reagent concentrations and working volumes are reduced.

In order to better show the performance of these new products, Corning conducted a series of experiments to evaluate these microplates with both different reagent concentrations and assay working volumes. We chose an enzymatic FP assay for these experiments, in which a fluorescently labeled substrate molecule is degraded over time by a protease enzyme. This causes changes in the substrate’s molecular rotation that is then reflected by a reduction in FP signal. The following products were evaluated:

- Corning 384 well low volume black medium bind assay plate
- Corning 384 well low volume black non-binding surface (NBS) coated assay plate
- Corning 1536 well black medium-bind assay plate
- Competitor’s 384 well small volume black medium bind assay plate

Materials and Methods

For FP assays, 1X digestion buffer and BODIPY FL casein substrate were diluted according to manufacturer instructions (Molecular Probes, Cat. No. E-6658) and dispensed into microplates of Corning 384 well LV black opaque MB, and Corning 1536 well black opaque MB assay plates (#3951) at a final substrate concentration of 100 pg/µL for 10, 5, and 1 µL working volumes. The final substrate concentration for 20 µL working volumes was 10 ng/µL. Dilutions (weight/volume) of Streptomyces griseus protease (Sigma® Cat. No. P-6911) in 1X digestion buffer were added to all but control microwells at varying concentrations (50 pg/µL, 25 pg/µL, 2.5 pg/µL, 0.25 pg/µL, and 0.125 pg/µL). Protease activity was detected in millipolarization units (mP) using the standard fluorescence polarization protocol in an LJL BioSystems Analyst™ (384 LV plates) and LJL BioSystems Acquest™ (1536 well plates), (Molecular Devices, Sunnyvale, CA) as follows: lamp = continuous, z-height = 1 mm, units = cps, attenuation mode = out, integration time = 100,000 µs, excitation filter = fluorescein 485 nm, emission filter = 530 nm, measurement type = comparator, sensitivity = 2, plate settling time = 150 ms, dynamic polarizer = emission, static polarizer = S, polarizer settling time = 30 ms. Detection instrument was programmed with appropriate plate dimensions for all plates. Corning 384 well Low Volume Plate Dimensions (in mm): length = 127.76, width = 85.47, rows = 16, columns = 24, well depth = 6.58, height = 14.22, row off-set = 8.96, column off-set = 12.11, row spacing = 4.5, column spacing = 4.5.

Results

Results from this FP assay for protease activity demonstrate, at 20 µL working volumes, Corning 384 well LV MB and NBS coated assay plates have significantly reduced FP signal (mP) compared to the competitor’s plates. This indicates significant enzymatic degradation of the fluorescently labeled substrate by the protease, at each protease concentration tested, at 10 minutes (Figure 1). Similar results were observed at 30 and 60 minutes (data not shown). This suggests that both Corning 384 well LV MB and NBS™ assay plates maintain assay integrity during working volume reduction to 20 µL, as well as reagent concentration reduction from 25 to 0.25 pg/µL.
When working at either 2.5 or 50 pg/µL protease concentrations in 10 µL working volumes, Corning® 384 well LV MB assay plates are comparable to the competitor’s small volume plates (Figure 2). However, the Corning 384 well LV NBS™ coated assay plates have significantly greater signal to noise ratios than the competitor’s plates under these conditions. This indicates that the NBS plates have performance capabilities for working with very low reagent concentrations that the other plates were unable to match.

When working at 0.125 pg/µL protease concentrations in 5 µL working volumes, the Corning 384 well LV MB and LV NBS coated assay plates have significantly greater signal to noise ratios than the competitor’s plates under these conditions (Figure 3). This indicates that both of the Corning plates are superior for working with very low reagent concentrations at volumes (5 µL/well) comparable to those found in 1536 deep well plates.

At working volumes of 1 µL and protease concentrations of 0.125 pg/µL, signal to noise ratios generated from this FP assay were significantly greater at 10 minutes in Corning 384 well LV NBS coated assay plates than Corning 1536 well MB assay plates or the competitor’s 384 Small Volume MB assay plates (Figure 4). This suggests that the round bottom shape and NBS coated surface of the 384 well LV assay plates are the basis for the superior performance of these plates that outper-

**Figure 1.** Dilutions of Streptomyces griseus protease was incubated with 10 ng/µL of BODIPY FL casein substrate for 10 minutes at room temperature in 20 µL volumes. Protease activity was detected by an LJL BioSystems Analyst™ as a reduction in mP units over time.

**Figure 2.** 50 pg/µL and 2.5 pg/µL of Streptomyces griseus protease was incubated with 100 pg/µL of BODIPY FL casein in 10 µL volumes for 10 minutes at room temperature. Protease activity was detected by an LJL BioSystems Analyst as a reduction in mP units over time. Signal-to-noise ratios represent DmP/average standard deviation.

**Figure 3.** 0.125 pg/µL of Streptomyces griseus protease was incubated with 100 pg/µL of BODIPY FL casein in 5 µL volumes for 10 minutes at room temperature. Protease activity was detected by an LJL BioSystems Analyst as a reduction in mP units over time. Signal to noise ratios represent DmP/average standard deviation.

**Figure 4.** 0.125 pg/µL of Streptomyces griseus protease was incubated with 100 pg/µL of BODIPY FL casein in 1 µL volumes for 10 minutes at room temperature. Protease activity was detected by an LJL BioSystems Analyst and LJL BioSystems Acquest™ as a reduction in mP units over time. Signal-to-noise ratios represent DmP/average standard deviation.
form both the competitor’s 384 Small Volume and Corning® 1536 well assay plates.

Conclusions
The demand for screening large compound collections against an increasing number of therapeutic targets has emphasized the need for assay miniaturization without sacrificing assay sensitivity. As stated in the introduction, low signal to noise ratios resulting from assay miniaturization may result in the loss of potential drug candidates during compound screenings. One approach to improve this undesirable side effect of assay miniaturization is to increase reagent concentrations; however, this solution can be costly. As shown by the results of the above experiments, a more viable approach is to reduce the loss of reagents from the reaction by non-specific adsorption to the walls as well as reduce fluid entrapment in the corners of flat bottom microwells. This approach can be accomplished by using the new Corning 384 well LV MB and NBS™ coated assay plates. These experiments demonstrated:

- Corning 384 well LV MB and NBS™ coated assay plates provide superior assay performance while working in low volumes (5 to 20 µL), as well as low reagent concentrations.
- Corning 384 well LV MB and NBS coated assay plates provide an alternative for those who wish to continue to work in the 384 well microplate format while working in very low volumes (1 to 5 µL), as well as very low reagent concentrations.
- Corning 384 well LV MB and NBS coated plates outperform the competitor’s small volume assay plate while working in low working volumes as well as reduced reagent concentrations, in a miniaturized homogeneous fluorescence polarization assay.

Acknowledgments
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Bibliography

Corning 384 Well Low Volume Microplates Ordering Information

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