Performance Advantage of Corning® NBS™ Microplates in Homogeneous Biochemical Assays

SnAPPShots
A brief report from the Corning Applications Group

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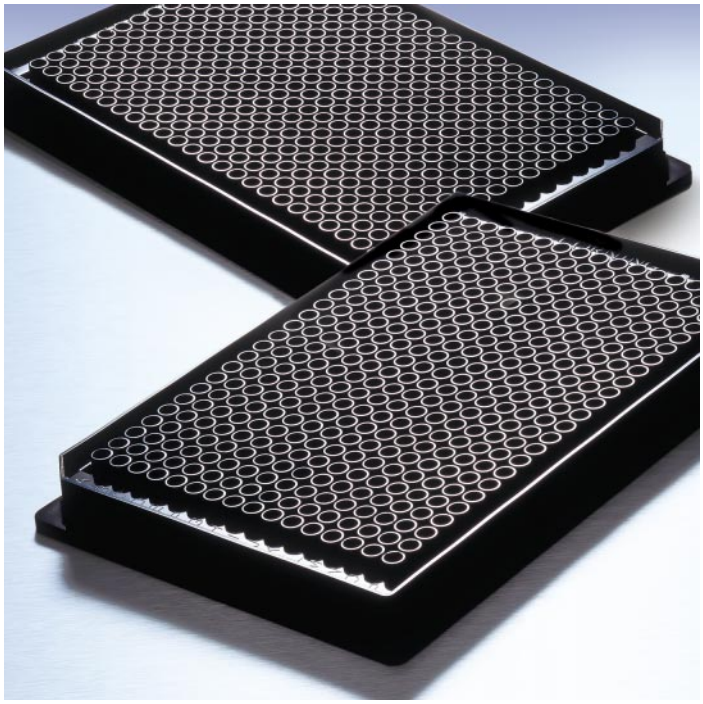

Introduction

The Corning proprietary NBS microplates provide an inert, nonionic and hydrophilic surface that minimizes molecular interactions. Previous studies[1,2,3] have shown that the NBS surface can drastically reduce non-specific protein binding to microplates, resulting in increased assay sensitivities and more consistent data. In the present study, we demonstrated that the NBS surface is essential to the success of certain types of HTS assays.

Method and Results

The experiments were performed using a novel fluorescence intensity based PKC kinase assay developed by Applied Biosystems, Inc. In this assay, the fluorescence of the fluorogenic substrate was quenched. Upon phosphorylation, the quenching mechanism is released, resulting in a significant increase in fluorescence intensity. Therefore, kinase activity can be monitored continuously by the change in fluorescence intensity of the enzyme reactions. The total reaction volume was 10 µL and the buffer contained 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 5 mM DTT, 10% Lipid Activator, 6 µM fluorogenic substrate, and 10 µM ATP. Fifty pg/µL of the active PKC bII was used for reactions containing enzymes.

Microplates used in the experiment included Corning® 384 well low volume (LV) untreated and NBS solid black microplates as well as 384 well low volume plates from two other manufacturers. Fluorescence signals were read by the LJL Acquest™ (Molecular Devices, Inc.) with excitation and emission wavelengths of 480 (20) nm and 530 (20) nm, respectively.

Data in Figure 1 show that the PKC assay provides a robust and linear measure of kinase activity when performed on NBS microplates (blue line). Nontreated polystyrene microplates only provided linear kinetic data for 10 to 20 minutes, and the signals of enzymatic reactions were never fully developed on these microplates (red, yellow and green lines in Figure 1). Further, background fluorescent signals, shown in Figure 2, were only found stable in NBS microplates (blue line). Significant losses of background fluorescent signals (35% within an hour of incubation) were observed on nontreated microplates (red, yellow and green lines in Figure 2).

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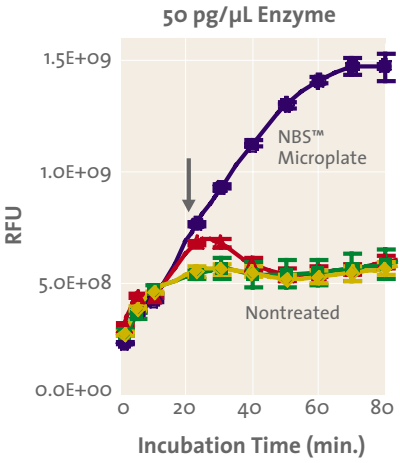

Figure 1. Comparison of PKC kinase signal development on nontreated and NBS microplates.

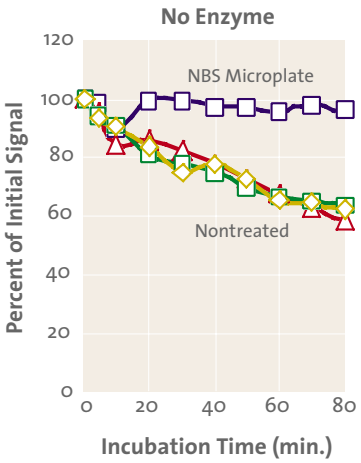

Figure 2. Comparison of background fluorescence stability on nontreated and NBS microplates.

Conclusions

- Corning® NBS™ microplates provide the widest signal dynamic range and most stable fluorescence signals for the PKC assay described in this report.
- This robust assay requires very little enzyme (50 pg/µL) to generate a significant amount of signal change within 1 hour of incubation.
- This simple and straightforward kinase assay technology is well suited for high throughput screening of inhibitors or substrates.

References

1. Binding Comparison of Polymer Surfaces: Introducing Nonbinding Surface Microplates
2. Corning Nonbinding Surface Microplates for Fluorescent HTS Assays
3. Corning Nonbinding Surface Treatment to Reduce Nonspecific Binding to Microplates

These three reports are available at: www.corning.com/lifesciences/technical/assay.

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