Performance Advantage of NBS™ Microplates in a Novel Fluorescent Intensity Based PKC Kinase Assay

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Overview

Purpose: To demonstrate the unique performance of NBS™ (an inert, low protein binding, nonionic and hydrophilic surface) microplates in a high-throughput PKC kinase assay developed by Applied Biosystems, Inc. Comparisons of the assay performance between NBS™ and nontreated microplates were also made.

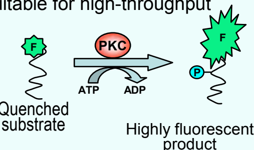
Methods: The kinase assay is a continuous fluorescent intensity based assay. The fluorogenic PKC substrate exhibits **6-fold increase** in fluorescence upon phosphorylation. Thus, PCK enzyme activity was monitored continuously over a 1-2 hr incubation period by looking at the increase of fluorescent intensity in the reactions.

Results: NBS™ is necessary for this high-throughput kinase assay in order to obtain linear kinetics and stable background fluorescent signal. Very little enzyme (50 pg/µL) is needed to generate significant amount of signal change within 1 hr of incubation. The NBS™ 384 Well Low Volume Microplates give excellent cross plate performance (Z' = 0.906). This kinase assay is simple and straight forward, making it extremely suitable for high-throughput screening of inhibitors or substrates.

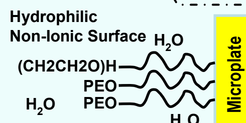
Introduction

❖ Kinase Assay:

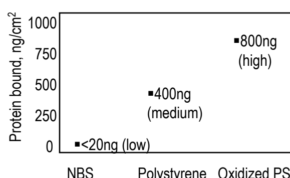
Traditionally, Protein Kinase activity was measured by monitoring product formation in a discontinuous manner. These methods are often lengthy and involve radioactive labeling or expensive antibodies. The ABI Kinase Assay used in this study monitors the phosphorylation of substrates continuously. The assay procedure is simple and straight forward, making it extremely suitable for high-throughput screening of inhibitors or substrates.



❖ NBS™ Mechanism:



❖ NBS™ drastically reduces protein binding



Materials & Methods

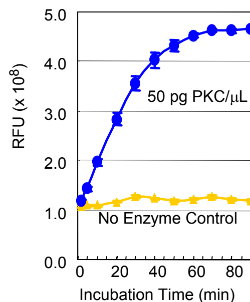
Materials

- ❖ Fluorogenic PKC substrate: Generous gift from ABI Inc.
- ❖ Kinase: PKC βII active unit from Upstate Biochemical
- ❖ NBS™ microplate: Corning catalog # 3676
- ❖ Nontreated polystyrene microplates: from competitors
- ❖ Microplate reader: LJL AcQuest (Molecular Devices, Inc.)

Procedure

- ❖ Make MasterMix (containing all the components necessary for PKC activity except for ATP)
- ❖ Add 1 µL ATP solution to each well
- ❖ Initiate the reaction in each well by adding 9 µL MasterMix
- ❖ Monitor fluorescent intensity chance at Ex/Em of 500/550 nm over 1-2 hr incubation period (RT: 21°C)

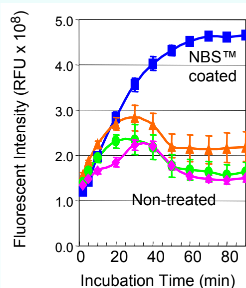
Results



Typical kinetics performed on NBS™ microplates:

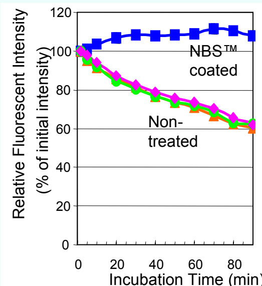
- ~ 3 - 4 times signal increase after 1 hr incubation in the presence of PKC
- Stable background fluorescent signals in the absence of PKC

(6µM substrate, 10µM ATP)



- Signal of kinase activity was ONLY enhanced and maintained throughout incubation time on NBS™ microplates.
- The enzymatic reactions were never fully developed on nontreated microplates

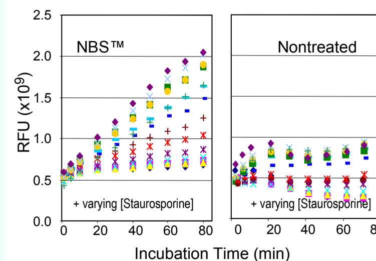
(6 µM substrate, 10 µM ATP, 50 pg/µL eznyme)

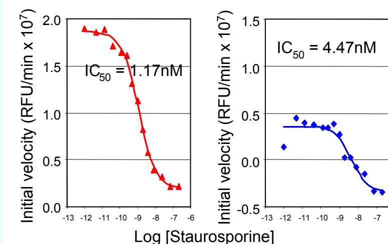


- Stable background fluorescent signals (from samples containing everything but PKC) on NBS™ microplate
- Significant loss (ca.30%/hr) of such signal on non-treated microplates

(The data are the average of 6 independent experiments.)

Inhibition of Staurosporine on PKC kinase Activity:

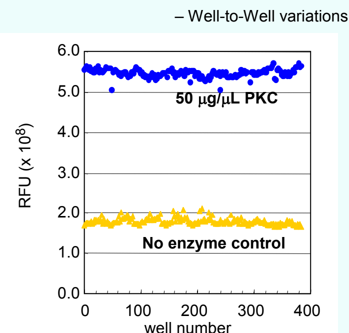




In drug inhibition assay, only NBS™ microplates generated acceptable inhibition curves (left side graphs), while nontreated microplates failed (right side graphs).

Cross Plate Performance:

– Well-to-Well variations



$$S/N = \frac{\text{Mean (max)} - \text{Mean (min)}}{\text{SQRT}(SD_{(Max)}^2 + SD_{(Max)}^2)} = \frac{381 \text{ mil}}{30 \text{ mil}} = 21$$

$$S/B = \frac{\text{Mean Signal}}{\text{Mean BLK}} = \frac{553 \text{ mil}}{172 \text{ mil}} = 3.2$$

$$Z' = 1 - \frac{[3{*}SD(max) - 3{*}SD(min)]}{[Mean(max) - Mean(min)]} = 0.906$$

- The graph shows the signals after 1 hr incubation on NBS™ microplates, and the calculation was based on data from 3 replicate plates.

Conclusions

- ❖ NBS™ microplates are necessary for this kinase assay in order to obtain linear kinetics and stable background fluorescent signals.
- ❖ Very little enzyme (50 pg/µL) is needed to generate significant amount of signal change within 1 hr of incubation
- ❖ NBS™ 384 well Low Volume Microplates give excellent cross plate performance (Z' = 0.906)
- ❖ The kinase assay is simple and straight forward, making it extremely suitable for high-throughput screening of inhibitors or substrates

** Acknowledgement: The kinase substrate was a generous gift from ABI Inc.