Optimizing a Corning® Epic® Biochemical Assay: An Example with the Lactate Dehydrogenase A

SnAPPShots

A brief technical report from the Corning Applications Group

Nicolas Andre, Ph.D.,
Sylvie Bailly
Corning Incorporated
7 bis Avenue de Valvins
Avon Cedex, France

Introduction

The Epic System is a high-throughput, label-free screening platform that allows for the observation of direct biomolecular interactions in a biochemical binding assay, and pharmacologically more relevant cellular responses to drugs in a cell-based assay. It consists of a disposable ANSI/SBS standard 384 well microplate with resonant waveguide optical biosensors integrated in each well, and an HTS-compatible optical reader. It eliminates issues such as background fluorescent interference to detection that is associated with the use of labels, while providing access to new biochemical and pharmacological information for drug discovery.

Assay development is a crucial step towards biochemical investigation and in drug discovery process. The Epic System, with its 384 well microplate and HTS-compatible reader, allows testing a large number of conditions in a limited time, and leads to reduced assay development time, decreasing work load and cost.

The following demonstrates the steps for optimization of an assay developed for the well-characterized lactate dehydrogenase A (37, 380 Da, pI = 8.38), a key enzyme involved in the last steps of anaerobic glycolysis. Furthermore, this activity level serves as a marker in opportunistic infections associated with HIV (1). To assess protein binding, we used its natural ligand, nicotinamine adenine dinucleotide (NADH, 663 Da).

Materials and Methods

Microplate Activation

Epic biochemical assays are performed on amine-coupling surfaces (Corning Cat. No. 5041 and 5046XX1). In this study, Epic microplates (Corning Cat. No. 5046XX1) were activated with 10 µL of 200 mM 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), 50 mM sulfo-N-Hydroxysulfosuccinimide (sNHS) (Pierce Cat. No. 22981 and 24510), and incubated for 30 minutes at room temperature. The microplates were subsequently washed 3 times with 20 µL H_2O with 384 well pipettor (CyBi®-Well).

Protein Immobilization

Protein immobilization was accomplished by adding 10 µL of LDHA protein (provided by AstraZeneca, UK) at the specified concentration in the buffer listed below (Table 1) using a 384 well pipettor (CyBi-Well), and incubating overnight at 4°C. The microplates were subsequently blocked with 150 mM borate buffer (pH 9.2, 200 mM ethanolamine) for ten minutes, and washed three times with assay buffer (50 mM Hepes pH 7, 300 mM NaCl, 1.5 mM $MgCl_2$, 1 mM DTT), and finally 15 µL assay buffer was added.

Table 1. Immobilization Buffers Used for pH Scouting.
Protein directly diluted into the corresponding buffer to 50 µg/mL prior to transfer to the Epic microplate (Corning Cat. No. 5046XX1).

Immobilization Buffers	pH
20 mM sodium citrate	4.5
20 mM sodium citrate	5.0
20 mM sodium acetate	5.5
20 mM sodium phosphate	6.5
20 mM sodium phosphate	7.0
20 mM sodium phosphate	7.5

CORNING
Epic® system

Binding Assay

The binding assay was run on a Corning® Epic® instrument. After 25 minutes thermal equilibration, a baseline read was taken on the Epic microplates with protein immobilized in assay buffer. In the next step, 15 µL of compounds were added to the microplates with a 384 well pipettor (CyBi®-Well), and then mixed 10 times. The final read was taken after 25 minutes thermal equilibration.

Results

Protein Immobilization

pH Scouting: In order to identify the best conditions for protein immobilization, a pH scouting analysis was performed using six different buffer solutions (pH values ranging from 4.5 to 7.5), and 50 µg/mL LDHA as the standard starting concentration (Fig. 1). The maximum immobilization level was obtained in 20 mM sodium acetate buffer (pH 5.5) with more than 2,000 pm.

Nevertheless, the maximum binding signal with 50 µM NADH was obtained when protein was immobilized at pH 7.5 (~15 pm). Therefore, the maximum immobilization level did not necessarily lead to the highest binding signal. The protein activity might be decreased when incubated at pH values lower than 6.5.

LDHA concentration optimization: Once the immobilization buffer was selected, different LDHA concentrations were tested ranging from 30 to 100 µg/mL for immobilization. The immobilization level observed remained constant from 40 to 100 µg/mL at ~2,000 pm, and started to decrease at 30 µg/mL (Fig. 2).

Binding

In order to obtain binding statistics and determine the affinity of NADH to LDHA, a dose response binding assay was performed on three different concentrations of immobilized protein (Fig. 3). NADH binding to LDHA was saturable and specific at all three concentrations tested. The affinity measured, ~12 µM, was in the range expected (5 µM) (2), and the maximum binding signal was ~15 pm.

Assay Robustness

The robustness of the assay was determined in the presence of 5% DMSO, the conditions required for high concentration ligand screening. To improve robustness, we also tested for the presence of Tween® 20 (0.01%).

Three microplates were tested in parallel with 64 wells per condition to assess the reproducibility of the assay. These results are summarized in Table 2.

In presence of DMSO, the ligand binding level reached 18 pm with low variability over the three microplates (less than 10%). The assay robustness (Z') was improved in the presence of 0.01% Tween 20 and was >0.5 for the three microplates.

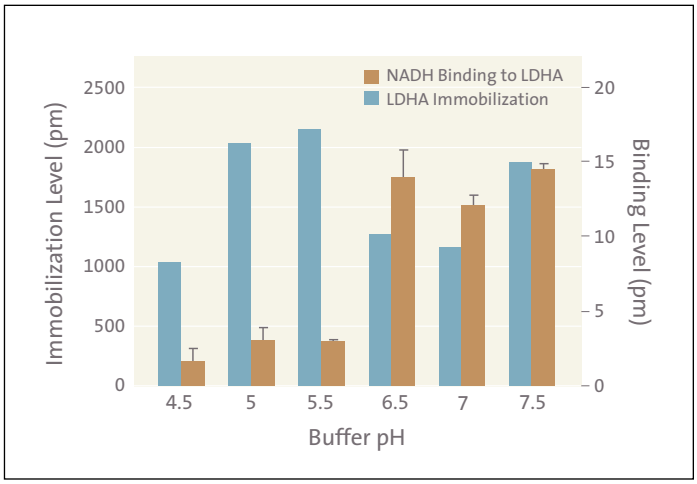

Figure 1. pH Scouting. Immobilization levels were monitored using different buffers with pH values ranging from 4.5 to 7.5 (50 µg/mL). The binding levels of 50 µM NADH is also monitored to ensure protein functionality.

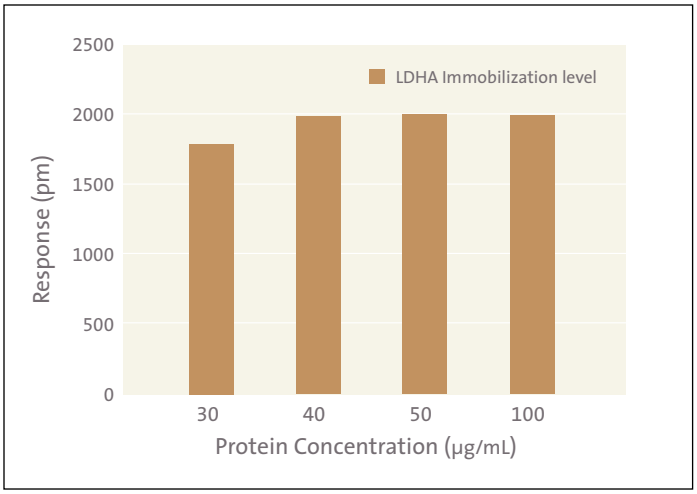

Figure 2. LDHA Concentration Optimization. Immobilization levels are monitored using different protein concentrations (30, 40, 50 and 100 µg/mL) in sodium phosphate buffer (pH 7.5).

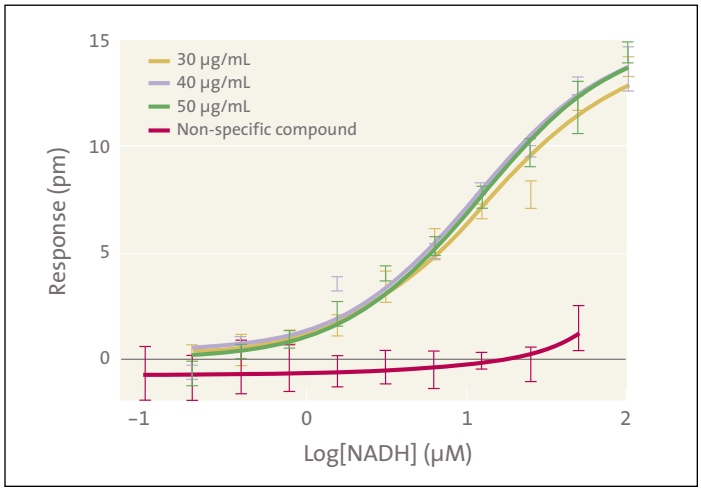

Figure 3. NADH Dose Response Curve. Specific binding of NADH to LDHA is monitored for protein immobilized at different concentrations (30, 40 and 50 µg/mL). The binding of a non-specific compound (red) to LDHA (50 µg/mL) is also monitored.

Conclusions

- The Corning® Epic® System enables label-free direct biochemical assay for small molecule binding to an immobilized protein. Several simple steps can be performed and are necessary to optimize the assay, including selecting the right pH and buffer conditions, optimizing concentration of target bound, and evaluating assay robustness with respect to buffer conditions.
- Applying the general strategy described here for most targets investigated can minimize the cost and time required for assay development.
- LDHA binding to NADH, evaluated using the Epic System after optimization of assay parameters, demonstrates high specificity to its natural ligand.
- Furthermore, the Z' was robust (>0.5), indicating that this assay is suitable for up-scaling for screening.
- Combining the high sensitivity and throughput, the Epic System is a reliable tool to be used in HTS biochemical assay for the drug discovery process.

Table 2. Assay Robustness Experiment Results. Experiment performed on three different microplates and conditions in 64 wells. Experiment performed with protein immobilized at 40 µg/mL.

3 times N = 64	Response (pm) at 100 µM NADH	CV (%)	Z'
No Tween® 20	18.9	9.0	0.4 ±0.13
0.01% Tween 20	18.6	9.7	0.55 ±0.3

References

1. Boldt MJ and Rai TR. Utility of lactate dehydrogenase vs. radiographic severity in the differential diagnosis of pneumocystis carinii pneumonia. CHEST 1997; 111:1187-1192.
2. Srivastava DK, Smolen P, Betts GF, Fukushima T, Spivey HO and Bernhard SA. Direct transfer of NADH between α-glycerol phosphate dehydrogenase and lactate dehydrogenase: Fact or misinterpretation? Proc. Natl. Acad. Sci. USA 1989; 86:6464-6468.

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 65 6733-6511
f 65 6735-2913

China
t 86-21-5467-4666
f 86-21-5407-5899

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 2-2716-0339

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

 Printed in USA 1/10 POD CLS-AN-133