TECHNICAL NOTE

Label-free Technology

Comparison of Performance: EnSpire Multimode Plate Reader and Corning® Epic® System

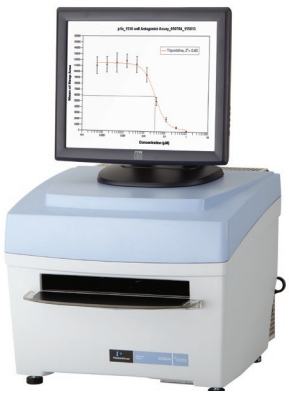
Authors

Miika Talvitie
Tim Cloutier, Ph. D.
Janet Park-Bewsher
Paul Butler

PerkinElmer, Inc.
Waltham, MA 02451
USA

INTRODUCTION

In recent years, label-free technology has gained momentum as an innovative tool in the fields of cellular research and biochemical binding assays. For cell-based assays, label-free offers distinct advantages including the recording of the integrated global response of cells, as well as the ability to monitor endogenously expressed receptors with high sensitivity. In biochemical assays it has the advantage over functional assays of recording actual binding events rather than the downstream functional effect of binding. Working with label-free assays also has some advantages over traditional labeled systems including the ability to avoid typical fluorescent or radioactive assay detriments, such as the potential to alter fundamental molecular function and complicated waste disposal. While fluorescent and radioactive detection technologies still dominate drug discovery and biological research worldwide, an increasing demand has emerged for label-free technology as a widely accepted research tool for mechanism of action (MOA) studies in lead optimization and receptor pharmacology, and as a robust system for monitoring protein:protein and protein:ligand binding events.



Corning Life Sciences' novel Epic® technology was established early on as the market leader delivering label-free technology to the research community. The Corning® Epic® System is a high-throughput label-free screening platform based on optical biosensor technology and performs both biochemical and cell-based drug discovery applications for a broad array of customers. This technology is now available on the EnSpire® Multimode Plate Reader from PerkinElmer, allowing the industry the ability to work with label-free assays, in a more affordable benchtop plate reader. This technical note will demonstrate the comparable performance of the EnSpire Multimode Plate Reader to the Corning® Epic® System.

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MATERIALS AND METHODS

Plate readers and detection technology

The Corning® Epic® System is a high-throughput label-free screening platform using patented optical biosensor technology.

The EnSpire combines the Epic® System's optical biosensor technology with traditional measurement technologies such as fluorescence, ultra-sensitive luminescence, and patented Alpha technology in a single benchtop unit. A resonant waveguide grating biosensor is incorporated into the bottom of each well of the proprietary label-free microplate. The EnSpire with label-free technology has a dedicated light source for exciting the microplate bottom. The EnSpire measures changes in local index of refraction. In cellular assays, these changes result from the ligand induced *dynamic mass redistribution* (DMR) within the bottom region (~150 nm) of the cell monolayer (cell-based assays). Binding events, such as an antigen binding to an antibody, create *mass change* in biochemical assays. These changes are detected as a picometer (pm) shift in the wavelength of the reflected light from the microplate. The peak position of the reflected wavelength spectrum is detected and analyzed using a spectrometer within the label-free technology module. The assay plates are measured before (the baseline read) and after (the final read) compound/sample addition. The results are reported as response (pm) showing the picometer shift between final and baseline reads. Response indicates DMR and mass changes in cell-based and biochemical assays, respectively.

Reagents for cell-based and biochemical assays

For reagents and detailed assay protocols see application notes 009449_01 and 009450_01.

Microplates

For cell-based assay protocols: EnSpire-LFC, 384-well (with fibronectin coating) microplates (Catalogue No. 6057428).

For biochemical assay protocols: EnSpire-LFB, 384-well (with amine-coupling) microplates (Catalogue No. 6057418).

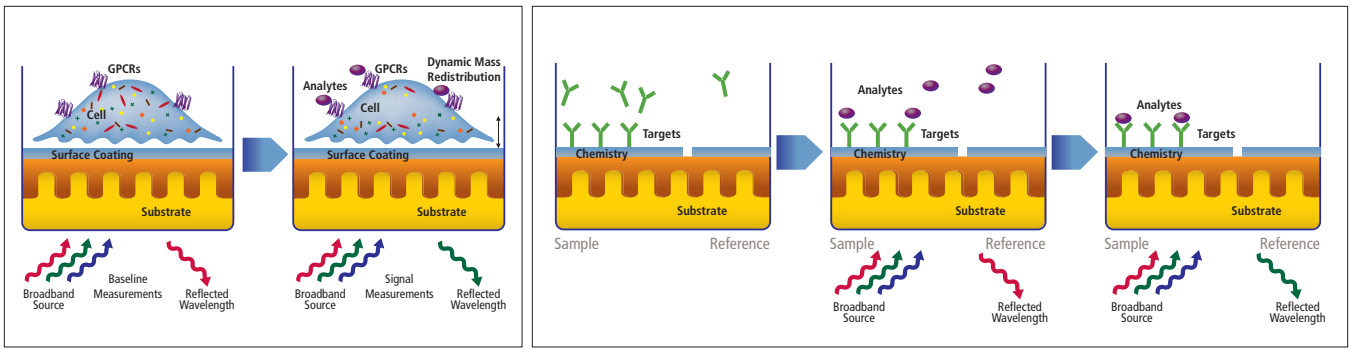
Cell-based label-free assay protocol:

1. Cell culturing & seeding to label-free plates
2. Buffer exchange (wash plate 3x)
3. Preparation of compound plates
4. Baseline measurement (4x)
5. Compound (antagonist) addition, 10 µl
6. Final reading, 30 repeats (antagonist read)
7. Compound (agonist) addition, 10 µl
8. 2nd Final read, 30 repeats (agonist read)
9. Data analysis using EnSpire software and GraphPad PRISM®

Biochemical label-free assay protocol:

1. Immobilization of protein
2. Buffer exchange, thermal equilibration 4 hours
3. Preparation of compound into label-free plates
4. Baseline measurement (4x)
5. Compound addition
6. Final reading, 4 repeats (antagonist read)
7. Data analysis using EnSpire software and GraphPad PRISM®

Label-free Assay Principles



Cell-based assays

Step 1: Grow cells overnight to get baseline measurement

Step 2: Add compound and measure signals

Biochemical assays

Step 1: Target is immobilized on the microplate amine-coupling surface

Step 2: Reference area prevents non-specific target immobilization followed by the washing and baseline read, and the addition of the analyte

Step 3: Analyte is bound to allow for final read

Data analysis

The integrated data analysis tools on the EnSpire were used to analyze the DMR (cell-based assay) and mass change (biochemical assay), and to generate dose response curves and subsequent calculations of EC/IC_{50}.

The following calculations were used to generate Response (pm) and EC/IC_{50} values:

1) Response (pm)
2) Response for selected plate selected repeat number. This calculation uses the result from the previous calculation as an input.
3) Generates dose response curves and reports EC/IC_{50} values. This calculation uses the result from the previous calculation as an input.

DISCUSSION

Label-free data obtained from cell-based and biochemical assays initiated on both platforms, suggest the performance of the EnSpire should be comparable to that of the Corning® Epic® System for these types of assays. Moreover, the data implies that current users of the Corning® Epic® System can expect comparable results when deploying their label-free applications on the EnSpire.

RESULTS

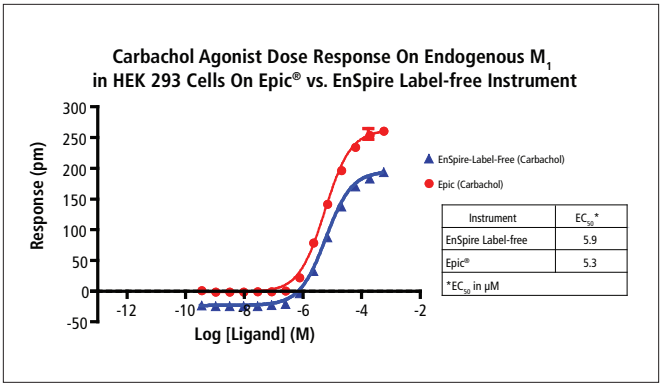


Figure 1 shows excellent correlation of the dose response profiles, as well as closely matched EC_{50} values for the cell-based assay.

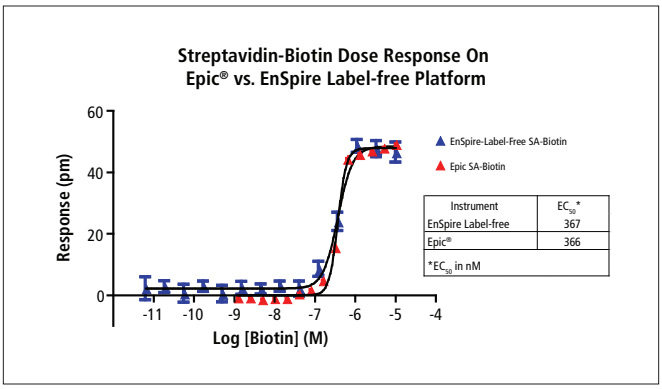


Figure 2 shows almost identical dose response profiles, as well as almost identical EC_{50} values for the biochemical assay.

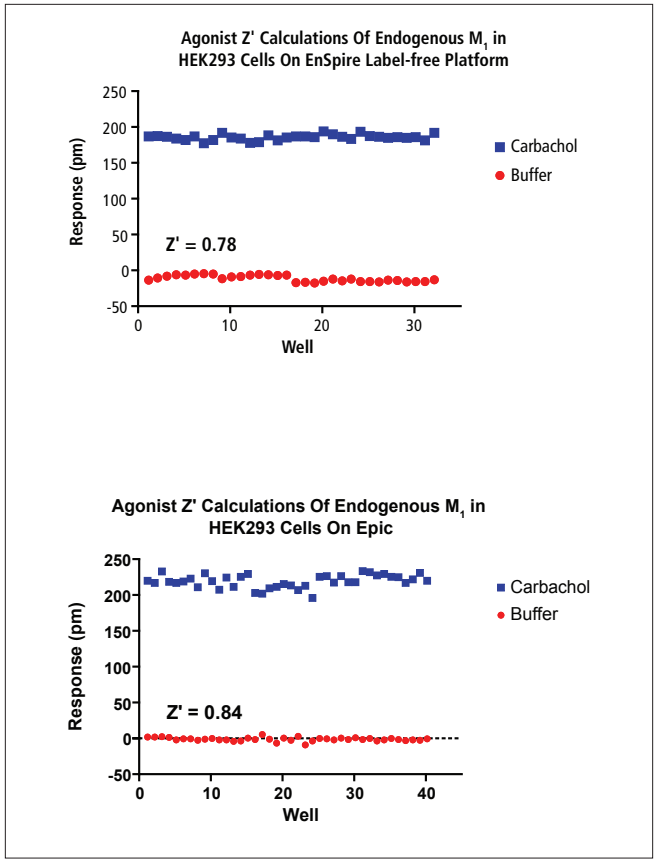


Figure 3 shows Z' values of 0.78 and 0.84 for the EnSpire label-free platform and Corning® Epic® platform respectively (i.e. cell-based assay) and demonstrates similar reproducibility of both systems.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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