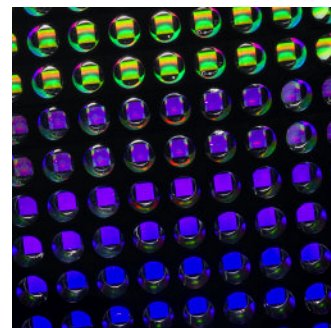


# Cell-Based Agonist and Antagonist Screens of the Endogenously Expressed $\beta_2$ AR in A431 Cells on the Epic<sup>®</sup> System



## SnAPPShots

A brief technical report  
from the Corning  
Applications Group

*Jeffery J. Scibek, Ph.D.  
Corning Incorporated  
Life Sciences  
Big Flats, New York*

## Introduction

G-protein coupled receptors (GPCRs) constitute the largest class of drug targets currently under therapeutic investigation. Most conventional technologies for studying GPCRs rely on the use of fluorescent labels, the bioluminescent photoprotein aequorin, or dyes to focus on a single component of a complex signaling pathway. Aside from the risk of introducing artifacts due to the labeled reagents, this approach most likely overlooks many of the pharmacologically relevant events that occur following receptor activation. In contrast, the Epic System provides a universal assay platform that is fundamentally pathway unbiased. The integrated response observed on the Epic System provides a more global view of the network interactions that occur within a cell during signal transduction.<sup>1</sup> This integrated response, along with a knowledge of chemical biology, enables researchers to identify coupling through all of the major GPCR classes ( $G_q$ ,  $G_s$ ,  $G_i$ ,  $G_{12/13}$ ). In addition, the sensitivity of the Epic System enables the detection of cellular responses from endogenous receptors without the need for engineering cells to over-express receptors of interest, thus eliminating the possibility of altering cellular biology and pharmacology due to receptor over-expression.

The A431 cell line was used as a model system to screen the Library of Pharmacologically Active Compounds (LOPAC) for agonists of endogenously expressed receptors and antagonists of the  $\beta_2$  adrenergic receptor ( $\beta_2$ AR). A431 cells are a human epidermoid carcinoma cell line that endogenously expresses the  $\beta_2$ AR. The LOPAC is a commercially available compound library that contains 1280 pharmacologically active compounds, including several known  $\beta_2$ AR agonists and one  $\beta_2$ AR antagonist.

A431 cells endogenously express the  $\beta_2$ AR in addition to a variety of other receptors on their surface. As a result, compounds that activate these other receptors can be identified as hits during the agonist screen. In essence, the agonist screen is a receptor-panning experiment, in which compounds that modulate any of the expressed receptors can be identified as a hit. In order to identify  $\beta_2$ AR antagonists, a known agonist (isoproterenol) was added to the cells that were previously treated with compounds from the LOPAC. A431 cells preincubated with compounds that are not  $\beta_2$ AR antagonists will be able to respond fully following the addition of isoproterenol. However, cells preincubated with a specific  $\beta_2$ AR antagonist, will not be able to respond following the addition of isoproterenol and no optical response will be observed. Since the Epic cell-based assay is noninvasive and nondestructive, agonist and antagonist screens can be performed in the same experiment.

## Materials and Methods

### Materials

A431 cells were purchased from ATCC<sup>®</sup> (CRL-1555). The LOPAC<sup>1280</sup> was purchased from Sigma-Aldrich<sup>®</sup> (LO1280). Isoproterenol (16504) and ICI 118551 (I127) were purchased from Sigma-Aldrich.

### Methods

#### *Preparation of A431 Cells for Agonist and Antagonist Screens*

A431 cells were detached from a nearly confluent T-150 flask (Corning Cat. No. 430825) using trypsin-EDTA. After detachment and counting, the cells were diluted in complete

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system

growth medium (DMEM + 10%FBS) and seeded at 20,000 cells/well (40  $\mu$ L volume) in an uncoated Epic<sup>®</sup> microplate (Corning Cat. No. 5040). The microplates were incubated overnight in a humidified, 37°C/5% CO<sub>2</sub> incubator. Following the overnight incubation, the complete growth medium was replaced with serum-free culture medium. A431 cells were then serum starved overnight in a humidified, 37°C/5% CO<sub>2</sub> incubator.

#### Agonist and Antagonist Screens on the Epic System

Following the overnight serum starvation step, the A431 cells were washed and incubated in 40  $\mu$ L of assay buffer (HBSS/20mM HEPES/0.6% DMSO) for 1.5 hours. For the agonist screen, a short baseline scan was taken, followed by the addition of compounds from the LOPAC (1  $\mu$ M final assay concentration). The optical response was measured for 60 minutes after compound addition. The antagonist screen was performed next by adding isoproterenol (1 nM assay concentration) to the A431 cells that were previously treated with compounds from the LOPAC. The response was monitored for 60 minutes following the addition of isoproterenol. During each compound addition step, 10  $\mu$ L of compound was added followed by 3 mix cycles.

### Results and Discussion

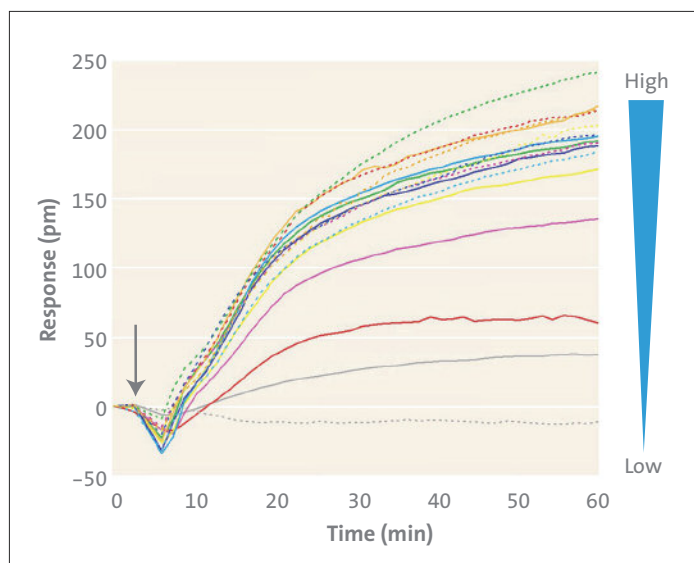
Figure 1 shows the kinetic profile for A431 cells following stimulation with a specific  $\beta_2$ AR agonist, isoproterenol. The Epic System measures the dynamic mass redistribution (DMR) that occurs within a cell following receptor activation. After isoproterenol addition (arrow), there is an immediate negative DMR phase that is followed by a positive DMR phase in which the response increases and then plateaus over time. This profile is consistent with the profiles observed for other G<sub>s</sub>-coupled receptors in various human cell lines, including A431. EC<sub>50</sub> values ranging from 0.05 to 0.1 nM were consistently observed.

#### Agonist Screen

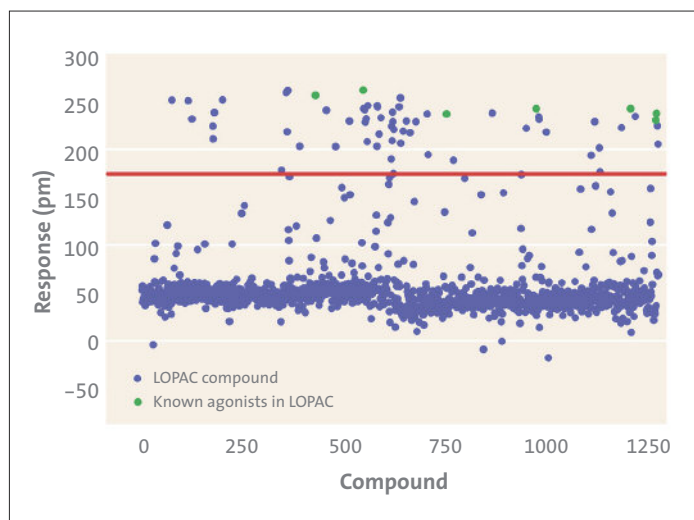
During the agonist screen, compounds from the LOPAC were screened at a final assay concentration of 1  $\mu$ M. In Figure 2, the response level for each of the 1280 compounds is plotted. In order to identify hits, a threshold was set based on the response of the positive control (EC<sub>95</sub> concentration of isoproterenol). For this screen, the threshold was set at 75% of the response of the positive control (~170 pm). Based on this threshold, 70 compounds were identified as hits in this screen. The LOPAC is known to contain 7 compounds that are classified as specific  $\beta_2$ AR agonists. Importantly, each of these compounds was positively identified as a hit (green data points), indicating that there were no false negatives in the screen.

#### Antagonist Screen

An important feature of cell-based assays on the Epic System is the ability to perform agonist and antagonist screens in the same experiment. On the Epic System, the agonist screen was performed by adding the LOPAC compounds at a single



**Figure 1.** Kinetic profile of the isoproterenol response in A431 cells. A431 cells were seeded on an uncoated Epic microplate. A dilution series of isoproterenol was added to the cells from 400 nM to 0.02 nM. Isoproterenol addition is indicated by the arrow. The Epic response is measured as a shift in reflected wavelength and expressed in picometers (pm). The response was monitored continuously for 60 minutes after isoproterenol addition.



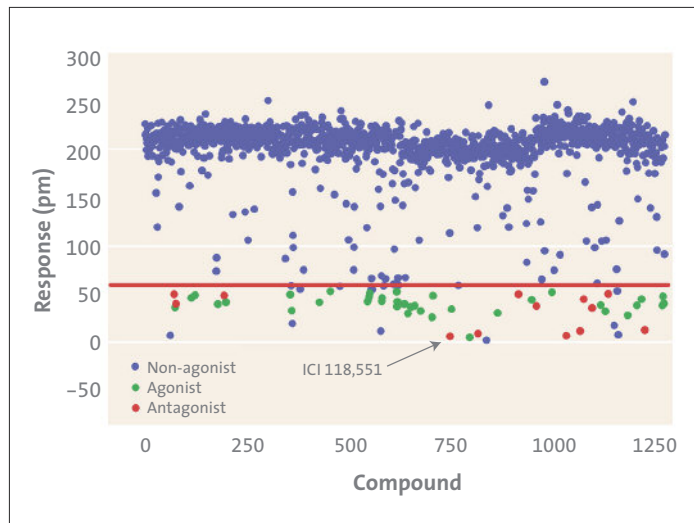
**Figure 2.** Representative agonist screen of the LOPAC using A431 cells. Compounds from the LOPAC were screened at 1  $\mu$ M. The response level was measured at 60 minutes after compound addition. The threshold was set at 75% of the response of the positive control (red line). Compounds in the LOPAC that are known  $\beta_2$ AR agonists are shown in green. The data point for thapsigargin (>500 pm) is not seen in this plot due to the y-axis scale.

fixed concentration (1  $\mu$ M) to the cells. After compound addition, the response was monitored for 60 minutes. Next, the antagonist screen was performed by adding isoproterenol (specific  $\beta_2$ AR agonist) to the cells that were pretreated with the compounds from the LOPAC. The cellular response was then measured 60 minutes after the addition of isoproterenol.

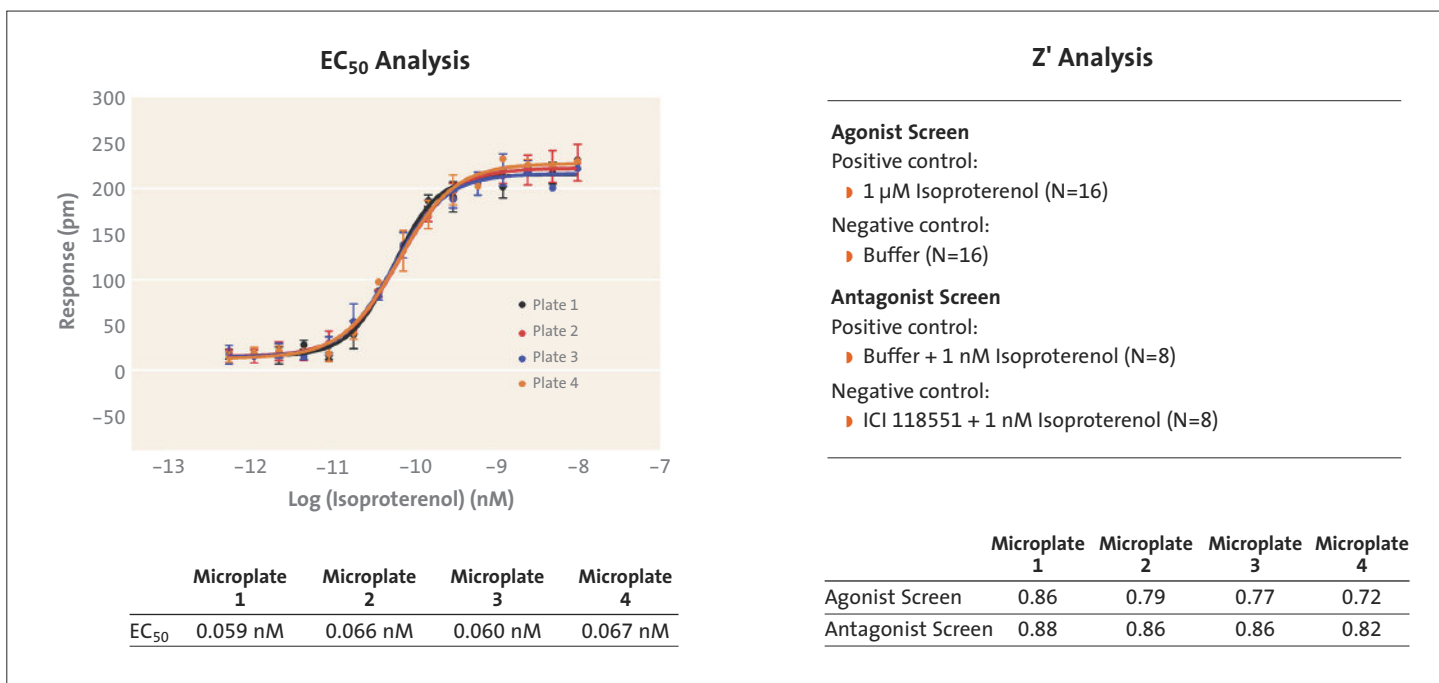
Figure 3 shows the results for a representative antagonist screen. During data analysis, the compounds were divided into three different groups: non-agonists, agonists, and antagonists. Most of the compounds were characterized as non-agonists (blue data points). These compounds were non-responders in the agonist screen and did not inhibit the isoproterenol response in the antagonist screen. Several of the compounds were characterized as agonists (green data points). These compounds were identified as full agonists in the agonist screen and also inhibited the isoproterenol response during the antagonist screen. Even though these compounds inhibited the isoproterenol response in the antagonist screen, they are not true antagonists. These compounds are able to inhibit the isoproterenol response due to receptor desensitization and not true antagonism. A small set of compounds was characterized as true antagonists (red data points). These compounds did not elicit a response during the agonist screen; however, they completely inhibited the isoproterenol response during the antagonist screen. Importantly, ICI 118,551, the only specific  $\beta_2$ AR antagonist in the LOPAC, was correctly identified as an antagonist in the screen.

### Assay Controls

Each Epic<sup>®</sup> microplate contained control wells in order to evaluate assay robustness and microplate-to-microplate consistency. Figure 4 shows the EC<sub>50</sub> values (left panel) and Z' values (right panel) for each of the Epic microplates from



**Figure 3.** Representative antagonist screen of the LOPAC using A431 cells. Compounds from the LOPAC (1  $\mu$ M) were added to the wells and the microplate was incubated for 60 minutes. Next, isoproterenol (1 nM) was added to the wells and the response was measured after 60 minutes. Compounds were classified as non-agonists (blue), agonists (green), or antagonists (red). Non-agonist: <25% response (non-responder) in agonist screen, >75% response in antagonist screen. Agonist: >75% response in agonist screen, <25% response (non-responder) in antagonist screen. Antagonist: <25% response (non-responder) in agonist screen, <25% response (non-responder) in antagonist screen. The black arrow highlights the compound ICI 118,551, a known  $\beta_2$ AR antagonist in the LOPAC.



**Figure 4.** EC<sub>50</sub> and Z' Analysis for A431 LOPAC screen. Left panel: Isoproterenol dose-dependent response and EC<sub>50</sub> analysis. Isoproterenol was added to each Epic microplate from 0.6 pM to 10 nM. The response was measured ~60 minutes after compound addition. Right panel: Z' analysis for the agonist and antagonist screen. ICI 118,551 is a specific  $\beta_2$ AR antagonist.

the screen. Importantly, the A431 cells exhibited similar EC<sub>50</sub> values on each microplate. Additionally, the Z' values exceeded 0.7 for each microplate in the agonist and antagonist screens. These results indicate that the cell-based Epic<sup>®</sup> assay with A431 cells is very reproducible and robust.

## Conclusions

- ▶ Agonist and antagonist screens can be run in the same experiment on the Epic System by employing a dual compound addition strategy.
- ▶ All of the known  $\beta_2$ AR agonists and antagonists were correctly identified; indicating that there were no false negatives.

- ▶ Similar EC<sub>50</sub> values were observed for isoproterenol on each Epic microplate, indicating good microplate-to-microplate consistency.
- ▶ Z' values exceeded 0.7 for each of the microplates, indicating that the Epic cell-based assay was very robust.
- ▶ Cell-based assays on the Epic System are non-invasive and non-destructive, allowing agonist and antagonist screens to be performed in the same experiment.

## References

1. Fang, Y., Ferrie, A.M., Fontaine, N.H., Mauro, J., Balakrishnan, J. (2006). Resonant Waveguide Grating Biosensor for Living Cell Sensing. *Biophys. J.* 91:1925-1940.

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## 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

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