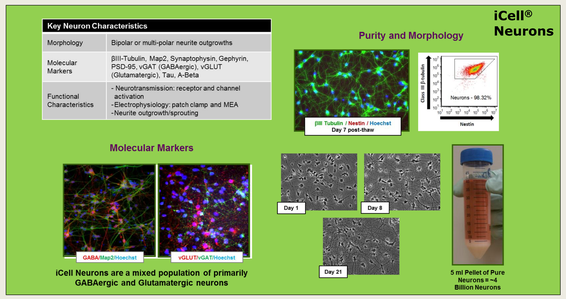
Label-Free Analysis of the endogenous $GABA_A$ Receptor in iPSC-Derived Neurons Using the EnSpire® Multimode Reader and JANUS® Automated Workstation

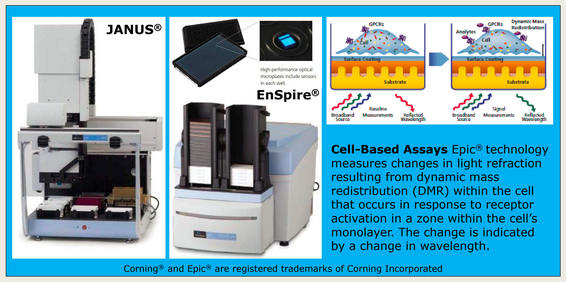
Heidi Morgan, Frauke Haenel, Maxine Santoro, Jennica Lutz, and Tim Henion – PerkinElmer, Inc., Waltham, MA USA
Coby Carlson, Rachel Llanas, and Arne Thompson – Cellular Dynamics International, Madison, WI USA

1 Introduction

The increased demand for simpler, more physiologically-relevant assay platforms has lead to the implementation of both label-free technologies and stem cells together in drug discovery research. Automated workflows are also needed to facilitate studies by increasing cell-based assay efficiency, reproducibility, and performance. To address these needs, methods utilizing the PerkinElmer EnSpire® Multimode Plate Reader with Corning® Epic® label-free technology and the JANUS® Automated Workstation to non-invasively and phenotypically analyze iCell® Neurons have been developed. These cells are derived from human induced pluripotent stem (iPS) cells and are a mixture of post-mitotic neuronal subtypes, composed primarily of GABAergic and glutamatergic neurons. iCell® Neurons display physiological functions and characteristics of normal human neurons, including the endogenous expression of various ion channels and GPCRs, providing a unique *in vitro* system for preclinical drug discovery, neurotoxicity testing, predictive disease modeling, and basic cellular research.

Here we present the application of label-free technology to study the endogenously expressed $GABA_A$ receptor, which is a ligand-gated ion channel. By monitoring the compound-induced dynamic mass redistribution (DMR) in iCell® Neurons, the EnSpire label-free platform detects the response in a "whole cell" format. These data demonstrate how to easily combine a novel phenotypic screening method with iPS cell-derived neurons, also with the added utility of automated liquid handling, to ensure robust and reproducible cellular assays. This approach lays the foundation for downstream interrogation of this cell model for neurodegenerative disorders such as Alzheimer's and Parkinson's Disease.

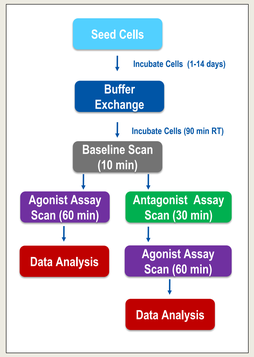




Cell-Based Assays Epic® technology measures changes in light refraction resulting from dynamic mass redistribution (DMR) within the cell that occurs in response to receptor activation in a zone within the cell's monolayer. The change is indicated by a change in wavelength.

2 Materials and Methods

Label-free Assay Workflow



Cellular label-free 384-well microplates (PerkinElmer, Cat. No. 6057400) were coated with a base layer of poly-L-ornithine (Sigma-Aldrich, Cat. No. P4957) and a top coating of laminin (Sigma-Aldrich, Cat. No. L2020) prior to cell seeding. The coated sensor plates were pre-wet with iCell® Neurons Maintenance Medium and centrifuged for 1 min at 800 RPM. iCell® Neurons (CDI, Cat. No. NRC-100-010-001) were seeded into sensor plates at a density of 10-20K cells/well. The plates were then covered with MicroClime® Environmental Lids (Labcyte, Cat. No. LLS-0310) and incubated at room temperature for 30 min prior to incubation in 5% CO_2 at 37 °C for 1-14 days. Fresh Maintenance Medium was exchanged every 2-3 days.

On the day of measurement, cells were washed four times in HBSS containing 20 mM HEPES ("Assay Buffer") using the JANUS workstation. Sensor plates were equilibrated for 90 min near the EnSpire instrument. An initial baseline reading (10 min) was taken prior to addition of any ligands. For antagonist assays, addition of GABAzine (Sigma-Aldrich, Cat. No. S106) in assay buffer was followed by a 30 minute kinetic measurement. Another addition of the agonist GABA (Sigma-Aldrich, Cat. No. A2129) in assay buffer was followed by a 60 minute final kinetic read. Compounds were dispensed as 5X stocks using the JANUS automated workstation.

The kinetic profiles were analyzed using the EnSpire software and dose response curves were generated using GraphPad Prism® software. For each compound, the difference between the last baseline measurement and the peak response following compound addition was calculated.

3 Assay Optimization: Cell Number Per Well

In order to determine the optimal signal in the assay, iCell® Neurons were seeded at three different densities and treated with a dose response of GABA on Day 5 post-thaw. The label-free response was monitored over 60 minutes on the EnSpire multimode plate reader, and the peak DMR values were used to generate concentration-response curves (and EC_{50} values) for each density. As shown in **Figure 1**, a robust response was observed at each density – with 20K cells/well providing the maximum signal. However, using lower cell numbers is acceptable and may provide an additional cost savings benefit when running this assay.

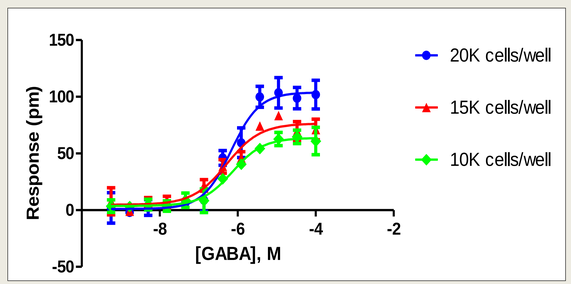


Figure 1. Dose-dependent responses of iCell® Neurons to the $GABA_A$ receptor agonist GABA on Day 5 of culture at three different seeding densities (20K, 15K, and 10K cells/well). EC50 values around 1 μM were determined at all 3 cell densities.

4 Modulation of the $GABA_A$ Receptor

In order to determine whether the EnSpire label-free can be used to detect a $GABA_A$-specific response, the chemical neurotransmitter gamma aminobutyric acid (GABA) was added to iCell® Neurons and the DMR responses were monitored. As shown in **Figure 2**, activation of the receptor with GABA was dose-dependent and saturatble with an EC_{50} value around 1 μM. This is in good agreement with literature values using other technologies such as ion flux assays or whole cell patch clamp recordings. In contrast, the antagonist GABAzine was able to inhibit the cellular response in the presence of GABA, showing this system is a good model for studying the $GABA_A$ receptor and related pathways.

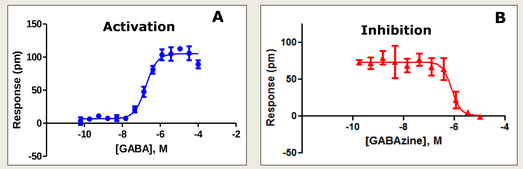


Figure 2. (A) Activation of the $GABA_A$ receptor in iCell® Neurons with GABA results in an EC_{50} value of $1.8x10^{-7}$ M; (B) Treatment of cells with a dose-response of GABAzine in the presence of 2 μM GABA (EC_{80} dose) attenuates the label-free response (IC_{50} value = $7.8x10^{-7}$ M). Both panels are representative of Day 5 culture and 15K cells/well.

5 Effects of Media and Time in Culture

In order to determine the optimal conditions for continued studies, GABA agonist assays were performed at two different days in culture, in both HBSS Assay Buffer and normal cell culture medium. As shown in **Figure 3**, the greatest response was observed on Day 5 in HBSS. The assay signal in HBSS was less at Day 14, but the iCell® Neurons still responded to GABA with a similar EC_{50} value. Interestingly, there was no response when analyzing GABA-induced DMR with cells in media. Reasons for these differences are still under investigation, but the label-free format has proven to be sensitive and efficient for studying these effects on neuronal plasticity.

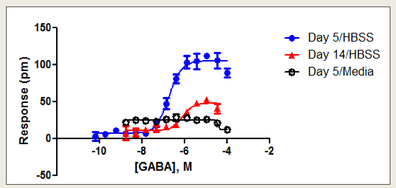


Figure 3. GABA dose-response with iCell® Neurons under three different conditions. The best assay signal was obtained on Day 5 in cultures with HBSS as the Assay Buffer for label-free readings.

6 DMSO Tolerance Studies

Most compound libraries are stored in DMSO, therefore HTS assays are generally performed in Assay Buffer containing small amounts of DMSO. In an effort to determine the amount of DMSO that can be tolerated by iCell® Neurons in the label-free assay, cells were seeded at 15K cells/well and assayed on Day 5 post-thaw. Titrations of GABA were added to the cells in Assay Buffer containing varied amounts of DMSO. **Figure 4** shows that the response and EC_{50} values stay consistent up to 0.5% DMSO, which makes this model highly amenable to screening campaigns.

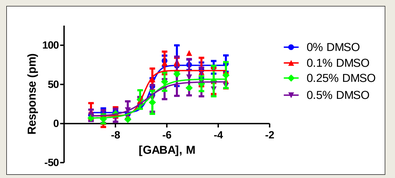


Figure 4. The label-free response of iCell® Neurons to GABA was tested for DMSO tolerance. Assay performance was generally consistent from 0% up to 0.5% DMSO in the Assay Buffer.

7 Validation of Assay Robustness

Modulation of endogenous receptor activity, such as for the $GABA_A$ ion channel receptor, requires that assay conditions be highly sensitive and robust. **Figure 5** demonstrates the high quality of the $GABA_A$ assays on the EnSpire label-free instrument. The Z' factor was determined by adding a stimulating dose of GABA (100 μM) in HBSS Assay Buffer to iCell® Neurons at an optimized cell density of 15K cells/well and measuring the peak DMR response over 60 minutes. Using 45 wells for maximum and 45 wells for minimum signal in the assay, the Z' factor was calculated and was shown to exceed 0.6. These data illustrate that the EnSpire label-free system can robustly perform stem cell screening assays.

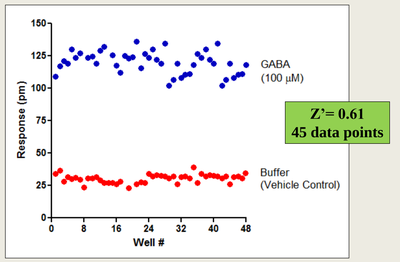


Figure 5. Data quality of $GABA_A$ assays with iCell® Neurons on the EnSpire instrument are excellent. Assay robustness was evaluated using a single concentration of GABA and the peak response was plotted (Z' >0.60).

8 Summary

An increased understanding of neurotransmitters in the brain and knowledge of the effects of drugs on the corresponding receptors comprise one of the largest research efforts in neuroscience. Scientists hope that this information will help them become more knowledgeable around the circuits responsible for disorders such as Parkinson's and Huntington's disease. The complexity of the nervous system makes it difficult to understand the underlying processes of neurodegeneration.

Simpler, "whole cell" assay formats combined with better disease modeling would significantly advance the understanding of these illnesses, ultimately leading to disease modifying drugs. Thus, iPS cells provide a biologically relevant model useful for drug discovery and toxicity testing, while the EnSpire label-free system is a non-invasive, highly sensitive tool used to detect internal cellular signaling changes.

By joining label-free with iPS cell technology, a powerful opportunity arises to develop more physiologically relevant cell-based assays for phenotypic screening, enabling the revolution of life-science research and personalized medicine.

- ❖ EnSpire label-free is a versatile instrument used for pathway-independent, global analysis of both basic and complex systems biology research and physiologically-relevant drug discovery
- ❖ Combination with the JANUS Automated Workstation leads to reduced cost, faster assay development, increased reproducibility, and simpler reagent requirements for stem cell research
- ❖ In collaboration with the high quality and fully optimized iCell® Neurons, EnSpire label-free is a sensitive system that easily detects endogenously expressed receptors, such as the $GABA_A$ subtype, which is an ideal target for new neurodegenerative therapies
- ❖ DMSO tolerance studies prove that iCell® Neurons respond well to agonist GABA up to 0.5% DMSO and the assay is shown to be highly robust with Z' factor of > 0.60
- ❖ Cell handling protocols, assay development procedures, and assay optimizations are readily available for iPSC screening campaigns utilizing EnSpire label-free technology