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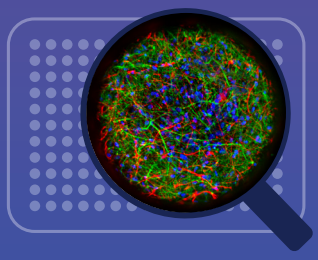
Application Note
microBrain® 3D Assay Ready



High Throughput Screening of LOPAC®1280 Using the microBrain® 3D Assay Ready Platform

Summary

- microBrain 3D Assay Ready is a highly consistent and scalable platform for high throughput screening studies
- 111 LOPAC®1280 compounds demonstrated neuromodulatory activity in microBrain spheroids at a single screening dose of 1µM
- microBrain 3D Assay Ready 384-Well Plates demonstrate high intra- and inter-plate consistency

Introduction

There is an increasing emphasis on using biologically relevant human neural cell-based assays in drug discovery to better mimic the complexity of the human brain and improve predictivity of *in vitro* drug screening assays. To this end, human induced Pluripotent Stem Cell (iPSC)-derived neural cells offer great potential for modeling neurological diseases and have found application in drug discovery for high content and phenotypic-based drug screening. However, lack of consistency limits their application for high throughput screening (HTS) and subsequent preclinical assay applications where low variability and high reproducibility are critical requirements for drug discovery and development.

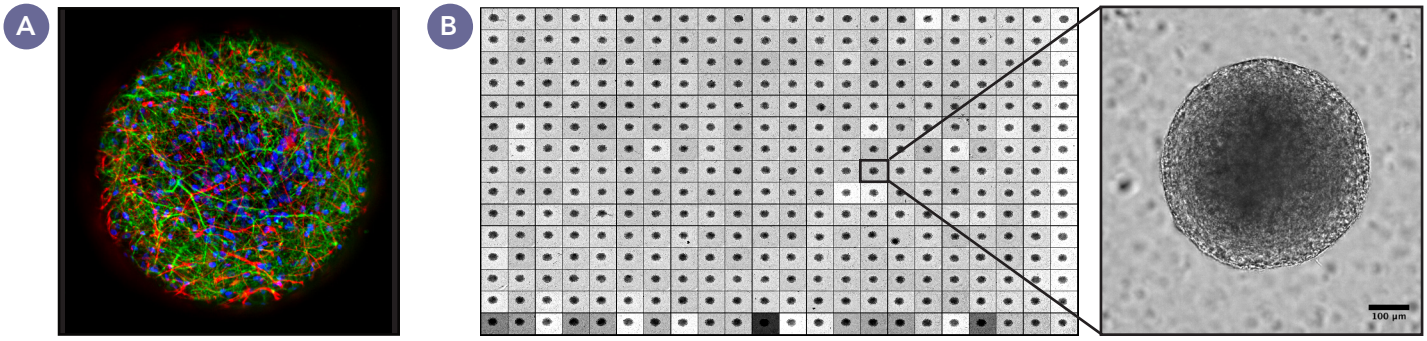
Here we describe an HTS-compatible 3-dimensional (3D) neural screening platform, the microBrain® 3D Assay Ready, comprised of a verified mixture of functional cortical glutamatergic and GABAergic neurons and astrocytes in uniformly sized mature neural spheroids (Figure 1A and 1B) in a 96- or 384-well format. microBrain spheroids present quantifiable, robust, and uniform spontaneous calcium oscillations that can be measured by kinetic fluorescence imaging, using high throughput systems such as the FLIPR and the ImageXPress (Molecular Devices). Moreover, the spheroids have been shown to be responsive to a broad spectrum of neuromodulators.

In this application note, we present the HTS capability of the microBrain 3D platform using Library of Pharmacologically Active Compounds (LOPAC®1280, Millipore-Sigma), a collection of 1280 compounds that cover a broad range of therapeutic activity.

Methods

Eight microBrain 3D Assay Ready 384-well plates (catalog #BSARX-AA-0384) were shipped overnight under ambient conditions by our standard method from Minneapolis, MN to the test site in San Diego, CA. LOPAC®1280 was purchased from Millipore-Sigma

Figure 1. (A) Immunostaining of a microBrain spheroid for MAP-2 (neurons) and GFAP (astrocytes) identified extensive distribution of both cell types throughout the spheroid. **(B)** Each well of the microBrain 3D Assay Ready 384-well plate contains a single spheroid matured to 8 weeks for this experiment.



(catalog #LO1280). Each of the 1280 LOPAC compounds were added to individual wells of the StemoniX microBrain 3D plates to a final concentration of 1µM and were tested in duplicate over two days. To measure the patterns and frequencies of the Ca^{2+} oscillations of the microBrain 3D spheroids, which were suspended in commercially available media, intracellular Ca^{2+} levels were measured at baseline and 30min, 2hr, and 4hr after compound addition. All measurements were performed for 10min using the FLIPR Tetra® High Throughput Cellular Screening System and the FLIPR Calcium 6 dye (Molecular Devices).

Results

Levels of variability both within and across plates were calculated for the control (DMSO) wells (64 wells per plate) to show the effect of the vehicle. Variability of spheroid activity for these control wells, expressed as percent standard deviation, was 14.8±0.8% across all tested wells. Similarly, the level of variability in peak count and peak spacing for all drug-tested spheroids, also expressed as Percent Standard Deviation, was calculated for all 1280 tested compounds (2560 total wells across 8 plates). Over 90% of all screened wells showed a variability level less than 20%, which is below the accepted range for cell-based assays. Over 50% of the compounds demonstrated extremely low variability levels less than 10% **(Figure 2)**.

Hits were identified based on how compounds impacted the peak count and peak amplitude of the measured calcium oscillation compared to the baseline measurement. A compound was selected as a hit if both replicates modulated peak count or peak amplitude by 2x the standard deviation of the control wells **(Figure 3)**. For peak count, this correlated with a 30% change; for peak amplitude, this correlated with a 40% change. Based on this paradigm, 111 hits were identified from the primary screen. Of these, 16 compounds scored for both peak count and peak amplitude across all time points, whilst 67 scored only for peak count or peak amplitude at a

Figure 2. StemoniX microBrain spheroids showed a high level of consistency across all LOPAC compounds and plates. The graph represents the percent standard deviation (St.DEV).

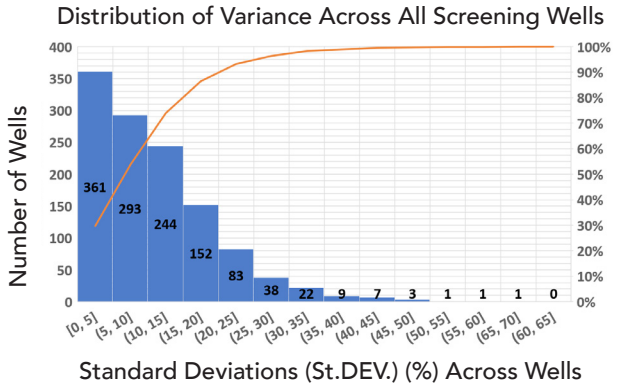
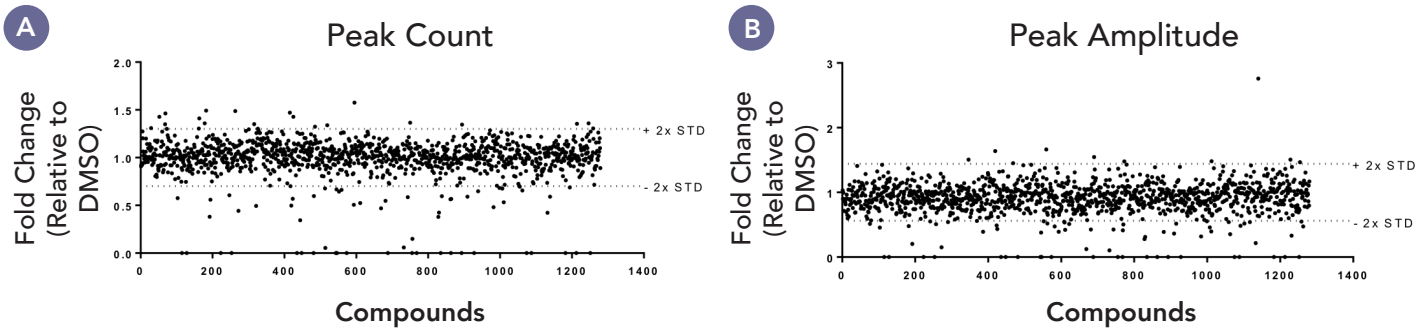


Figure 3. Dot-plot of total compound screen with cutoffs representing 2x-standard deviation (St.Dev.). **(A)** Peak Count. **(B)** Peak Amplitude. Of all tested compounds, 16 scored for both peak count and peak amplitude.



single time point.

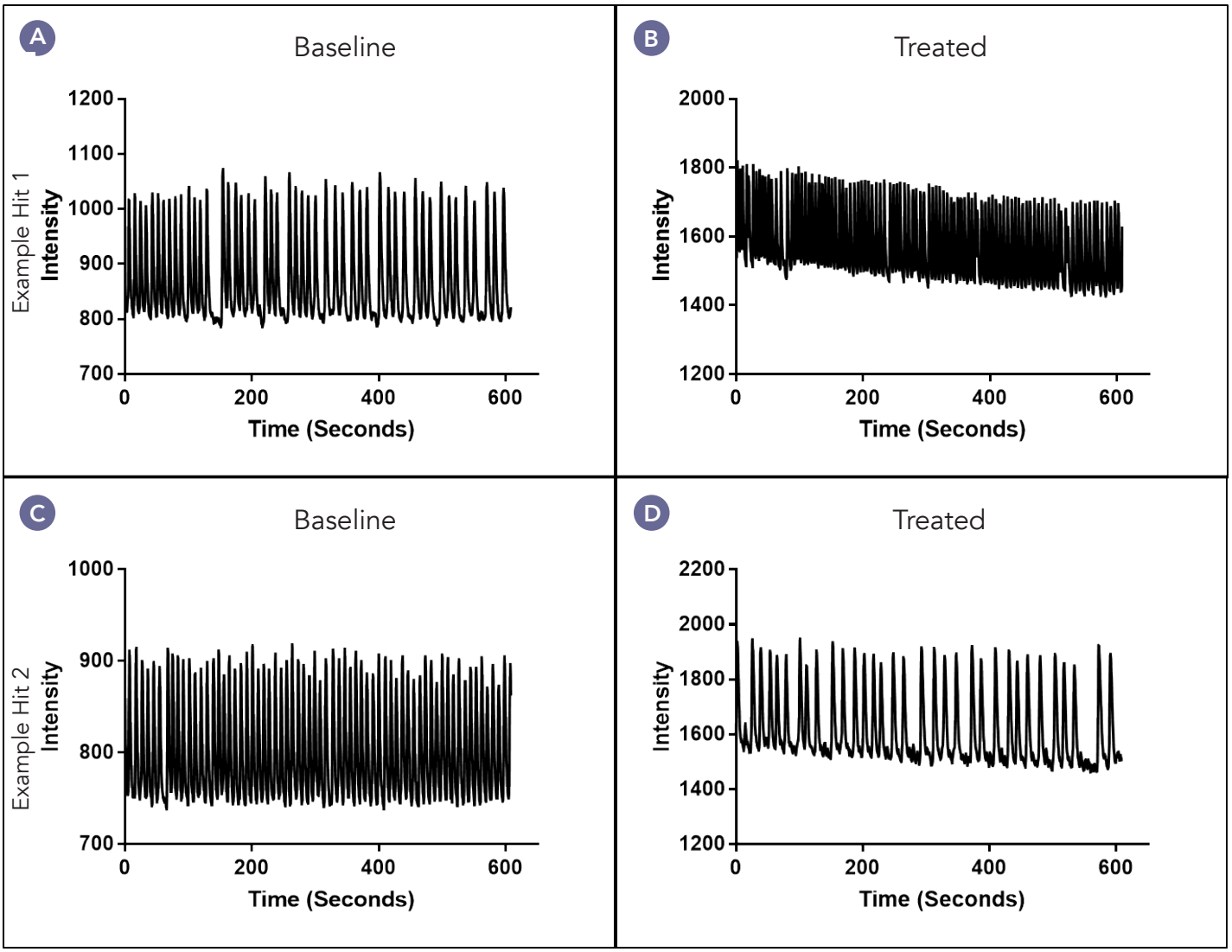
Results of two LOPAC®1280 compounds that demonstrated activity in microBrain 3D are shown in **Figure 4**. Analysis of calcium signaling by spheroids treated with Compound 1 showed a noticeable increase in peak count and decrease in peak spacing compared to baseline (pre-compound) activity **(Figure 4A and 4B)**. Conversely, analysis of calcium signaling by spheroids treated with Compound 2demonstrated a noticeable decrease in peak count with an accompanying increase in peak spacing compared to baseline activity **(Figure 4C and 4D)**.

Summary

LOPAC®1280 is a convenient compound collection perfect for validating the sensitivity, consistency and manipulability of the StemoniX microBrain 3D platform. Our results demonstrate that the StemoniX microBrain 3D Assay Ready system is a paragon for neuromodulatory studies and provides a highly consistent and scalable high throughput screening platform for large compound libraries that can identify novel therapeutics for subsequent development.

This work was done in collaboration with Janssen Pharmaceuticals.

Figure 4. Effects of 2 selected compounds in the LOPAC library on calcium oscillations in microBrain spheroids. Calcium oscillations are shown before **(A, C)** and after **(B, D)** compound addition.



microBrain® 3D Product Information

Product Name	Catalog #
microBrain® 3D Assay Ready 96-Well Plate	BSARX-AA-0096
microBrain® 3D Assay Ready 384-Well Plate	BSARX-AA-0384

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