StemoniX

Enabling Medicine to Work the First Time

Technical Summary
Functional Evaluation of microBrain® 3D Assay Ready Plates

Functional human iPSC-derived screening systems can recapitulate the human response for predictive results that avoid extrapolation across species. The microBrain 3D platform is comprised of neurologically active spheroids of human iPSC-derived neurons and astrocytes. Provided in high-throughput, ready-to-use formats that exhibit native human neurological responses, they provide an easy-to-use solution that accelerates *in vitro* disease modeling, screening, and toxicity testing.

microBrain 3D spheroids contain cocultures of neurons and astrocytes and are provided in 96- or 384-well formats.

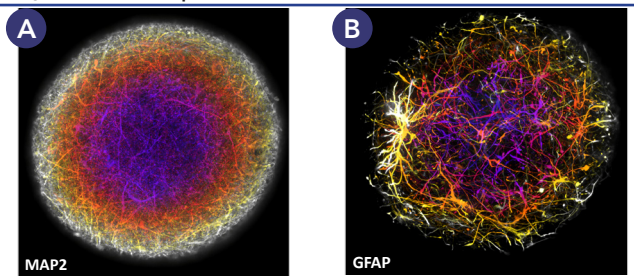

Figure 1. microBrain 3D spheroids are comprised of a coculture of (A) neurons (MAP2) and (B) astrocytes (GFAP) shown here as a pseudo-colored z-stack.

microBrain 3D spheroids are highly consistent across wells, columns, and rows.

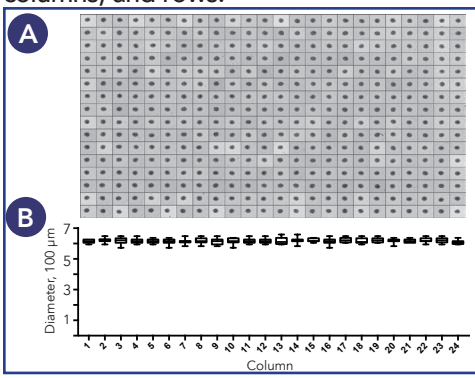

Figure 2. (A) Brightfield image of a 384-well microBrain 3D plate showing a single spheroid in each well. (B) Sample spheroid size distribution. CV<10%

Neuronal electrophysiology can be monitored and interrogated in high throughput via Ca^{2+} oscillations

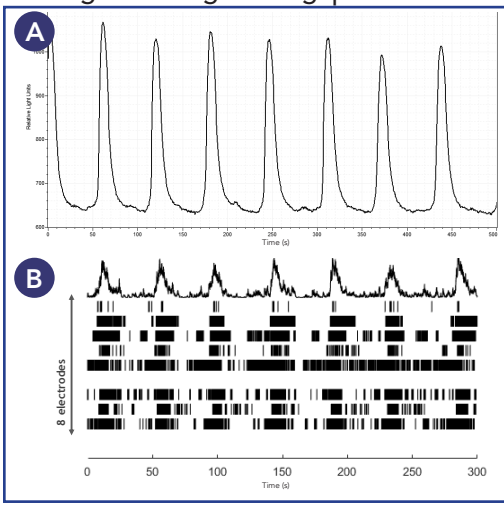

Figure 3. (A) Example Ca^{2+} oscillation trace monitored in real time and high throughput. (B) Example MEA raster plot showing neuronal oscillation behavior mirroring the Ca^{2+} oscillation.

microBrain 3D spheroids exhibit key responses to reference compounds.

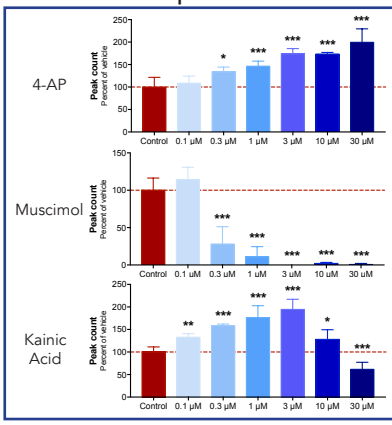

Figure 4. Changes in Ca^{2+} oscillation frequency to application of K^+ channel antagonist (4-AP), GABA agonist (Muscimol), and Kainate receptor antagonist (Kainic Acid); note dose dependent increase followed by toxicity-mediated decrease.

microBrain 3D serves as a powerful CNS-based screening platform for interrogating neural response across a wide variety of potential target pathways.

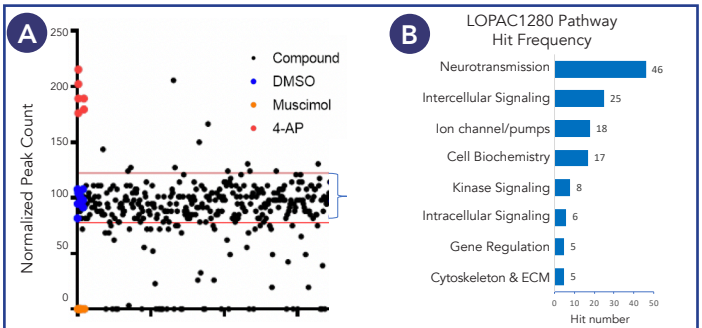

Figure 5. (A) Example data from a single screening plate showing the max and min response window, tight vehicle control response, and test compound effects. (B) Hit distribution across a wide span of neural and cellular biology.

microBrain 3D

- *Provides* a relevant and interrogatable human CNS platform for drug discovery.
- *Demonstrates* spontaneous and quantifiable calcium oscillations arising from neuronal activity.
- *Accelerates* CNS-based drug discovery with robust phenotypic screening.

For more information on functional interrogation and uses of the microBrain 3D platform, please visit StemoniX.com or contact us at info@stemonix.com

EXT-1702-02

Example Active Compounds	Mechanism of action
Acetylcholine chloride	Nicotinic and muscarinic acetylcholine receptors agonist
Altanserin	5-HT2A receptor antagonist (serotonin)
4-Aminopyridine	Non-selective KV channel blocker
(S)-AMPA	Selective AMPA agonist
(R)-Baclofen	Selective $GABA_B$ agonist
Benzbromarone	TMEM16A/B calcium-activated chloride channel (CaCC) blocker
Bicuculline	Potent $GABA_A$ antagonist
Biperiden HCl	Muscarinic receptor antagonist that displays some selectivity for the M1 subtype
Caffeine	Antagonist at A1 and A2A adenosine receptors; inhibitor of cyclic nucleotide phosphodiesterases
CI 966 HCl	Selective inhibitor of the GABA transporter GAT-1
CNQX	Potent AMPA/kainate antagonist
CPPG	Potent group III mGlu antagonist
Cyclothiazide	Positive allosteric modulator of AMPA receptors
DIDS	ClC-Ka chloride channel blocker
Dihydrokainic acid	EAAT2 (GLT-1)-selective non-transportable inhibitor of L-glutamate & L-aspartate uptake
Diphenhydramine HCl	H1 receptor antagonist
DNQX	Selective non-NMDA receptor antagonist
DOI HCl	Mixed 5-HT2A/2C agonist
Dopamine HCl	Endogenous agonist at dopamine D1-5 receptors
Eact	TMEM16A (ANO1) calcium-activated chloride channel (CaCC) activator; transient receptor potential vanilloid 1 (TRPV1) channel activator
Flupenthixol diHCl	Dopamine receptor antagonist (D1-5 R)
FPL 64176	Potent activator of L-type Ca^{2+} channels
GABA	Endogenous agonist GABA receptor; Endogenous inhibitory neurotransmitter
GABAzine	$GABA_A$ receptor antagonist
Gabapentin	Anti-epileptic like lamotrigine
Glycine	Add to NMDA or Ag NMDA glycine site
Glutamate	Brain's main excitatory neurotransmitter
L-glutamic acid	Endogenous, non-selective agonist glutamate receptor
Haloperidol	D2 Antagonist and NMDA sub Antagonist
Histamine diHCl	Endogenous histamine (H1-4) receptor agonist
Ibotenic acid	Potent agonist of the NMDA and group I (mGluR1 and mGluR5) and II (mGluR2 and mGluR3) metabotropic glutamate receptors.
Ifenprodil	NMDA 2B agonist
Isoproterenol HCl	Standard selective β-adrenoceptor agonist
Kainic acid	Selective agonist at kainate receptors
Lamotrigine isethionate	Na-channel blocking anti-epileptic; inhibitor of glutamate
Lidocaine	Na^+ channel blocker
Metergoline	5-HT2 antagonist. Also 5-HT1 antagonist and 5-HT1D ligand.
MK-801	Selective and non-competitive NMDA receptor antagonist
Muscimol	Potent $GABA_A$ agonist
NBQX	Potent, selective, and competitive AMPA receptor antagonist.
NMDA	NMDA agonist; Non-Specific
Noradrenaline bitartrate	Endogenous adrenergic hormone and neurotransmitter
Ouabain	Selective Na^+, K^+-ATPase inhibitor
(R)-(-)-Phenylephrine HCl	α1-adrenoceptor agonist
Phenytoin (dilantin)	Anti-epileptic like lamotrigine
Picrotoxin	$GABA_A$ receptor antagonist
Quinolinic acid	Endogenous NMDA agonist and transmitter candidate
Serotonin HCl	Endogenous agonist at 5-HT receptors and endogenous substrate for 5-HT transporters
SR 95531 HBr	Selective, competitive $GABA_A$ receptor antagonist.
TC-N 22A	Potent and selective mGlu4 receptor positive allosteric modulator
Tetrodotoxin	Na^+ channel blocker
Thioridazine HCl	Dopamine receptor antagonist; anti-psychotic
Verapamil HCl	L-type calcium channel blocker
Veratridine	Na^+ channel opener
WIN 55,212-2 mesylate	Highly potent cannabinoid receptor agonist