NeuCyte Labs

Translatable Neuroscience

Highly Functional iPSC-Derived Induced Neurons

Drug Discovery, Efficacy and Safety Assessment Services

Bridging the Drug Discovery Path with Translatable Neuroscience

The high attrition rate of novel CNS drugs during clinical development has been a major challenge to the pharmaceutical industry. This is largely attributed to the lack of biologically relevant models to study functional links between target and phenotype. NeuCyte's mission is to accelerate and optimize CNS drug discovery by developing more predictive assays and platforms for phenotypic screening.

Based on the advantageous SynFire® technology for generating human induced pluripotent stem cell (iPSC)-derived induced neuronal cells (iNs), NeuCyte has developed a proprietary in vitro human neural platform for complex electrophysiological and morphological readouts suited for target identification and validation, efficacy testing and neurotoxicity assessment. Using patient-derived, genetically and engineered defined neural cell types, NeuCyte builds unique cell-based assays for modeling neurological and neurodegenerative disorders.
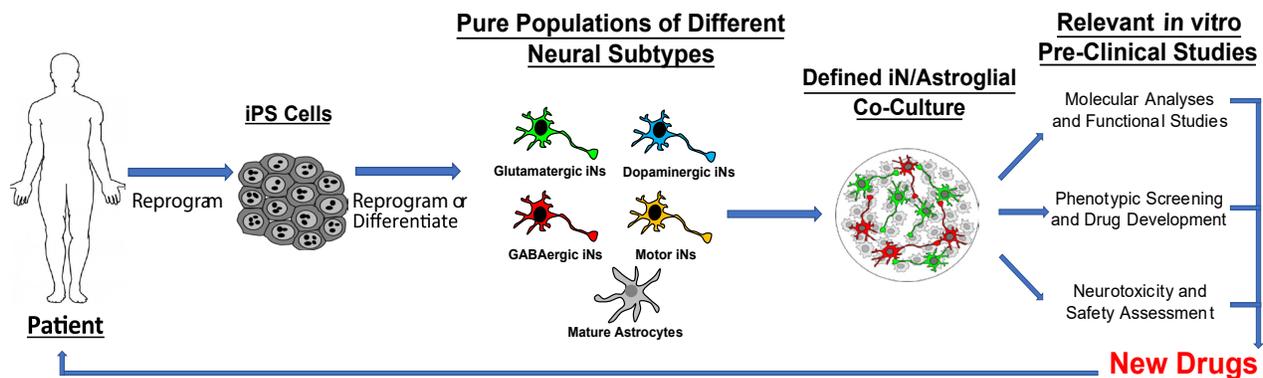

Figure 1. How NeuCyte can support neurological drug discovery and pre-clinical studies

NeuCyte Labs is the product and service division of NeuCyte. NeuCyte Labs offers:

Highly functional products and high quality services: NeuCyte Labs provides pure and ready-to-use iPSC-derived glutamatergic or GABAergic induced neurons (iNs) and astroglia. This platform most closely resembles real human neurobiology, providing the ability to effectively and confidently study the function of human neurons in vitro. Our services based on this platform are conducted by scientists who understand the system the best.

Extensive neuroscience expertise: NeuCyte Labs has put together an outstanding and focused scientific team. Our extensive knowledge of the biology behind human neurological disorders allows us to introduce advancements in in vitro disease modeling, particularly for phenotypic and target-based drug screens.
As our client, you always work directly with the neuroscientists who developed our technology platform, with no barrier in between.

Personalized approach towards each project: Our versatile in vitro cell system is suitable for compound efficacy screening and nonclinical neurotoxicity-based safety assessment for drugs and environmental chemicals. Our goal is to support our clients' needs using our technology platform. We always start with the questions you are trying to answer and design our work around your project.

Unique Enabling Technology

SynFire® iNs are generated using a patented procedure for direct reprogramming and exhibit the main characteristics of human primary neurons, such as expression of typical pan-neuronal markers and complex electrophysiology, including spontaneous/evoked action potentials and synchronized network activity. Neuronal subtype identities have been confirmed by staining and patch clamping.

SynFire iNs are suitable for a variety of functional assays. For example, the effect of compounds on neuronal survival, axonal outgrowth, or dendritic arborization can be measured by standard assessment of viability, or image-based analysis of labeled cells, respectively. When co-cultured with glial cells, effects on synapse formation and composition, transcriptional programs, and electrophysiology can be tested. Neuronal subtypes can be mixed in different ratios for making a defined co-culture for different experimental purposes.

Advantages of SynFire iNs include:

Real human biology: These cells more closely resemble real human biology than commonly used animal models and many other iPS-based systems, resulting in better suitability to predict responses to compounds.

Rapid and homogeneous maturation: SynFire iNs exhibit mature synaptic network activity within three to four weeks, such as synchronous bursting phenotypes silimar to those in rodent primary cultures.

Reliable, robust and ready-to-use: This reprogramming approach also results in a highly defined in vitro system and lot-to-lot consistency, providing reproducible results.
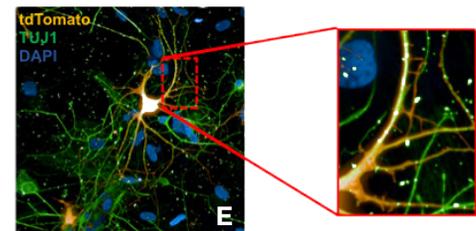
Flexible modular system: The user can control subtype to subtype relative seeding density and ratio, in order to track, analyze and manipulate specific cell types to fit individual projects.
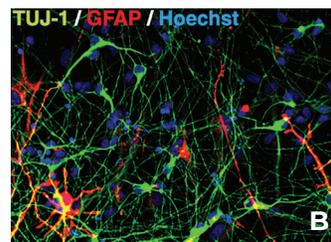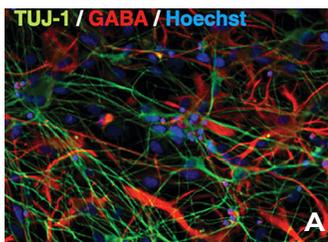
Pure populations of human neural cell types we offer:

- Glutamatergic excitatory neurons
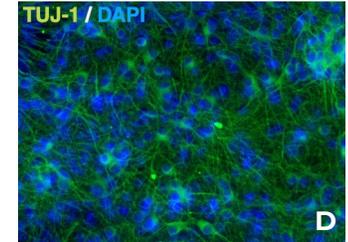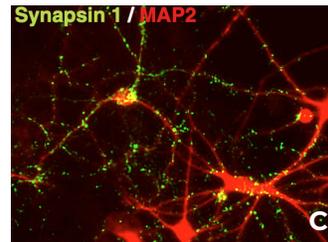- GABAergic inhibitory neurons
- Astroglia

Complex Morphologies


Pan-Neuronal and Subtype Specific Markers

Elaborate Networks

**Figure 2. SynFire iNs exhibit mature neuronal characteristics through immuno-staining**
SynFire iNs express pan-neuronal and subtype specific markers, rapidly mature to form complex networks and cellular morphologies. The modular aspect of SynFire neural cells allow for defined co-culture conditions and specific ratios of mixed neuronal subtypes, including inhibitory GABAergic neurons. (A) Pan-neuronal marker β3-Tubb (Tuj1) / Inhibitory neuron GABA-A neurotransmittor, α1 / Nuclear staining Hoeschst. (B) Pan-neuronal marker Tuj1 / Astroglia marker GFAP / Nuclear staining Hoeschst. 3-4 week old co-cultures exhibit complex neuronal networks, morphologies and show mature synaptic markers. (C) Pan-neuronal marker Map2 / Synaptic marker Synapsin1 / Nuclear staining Dapi. (D) Pan-neuronal marker Tuj1 / Nuclear staining Dapi. (E) Zoom in of spine-like formations on tdTomato and Tuj1 labeled glutamatergic excitatory neuron.
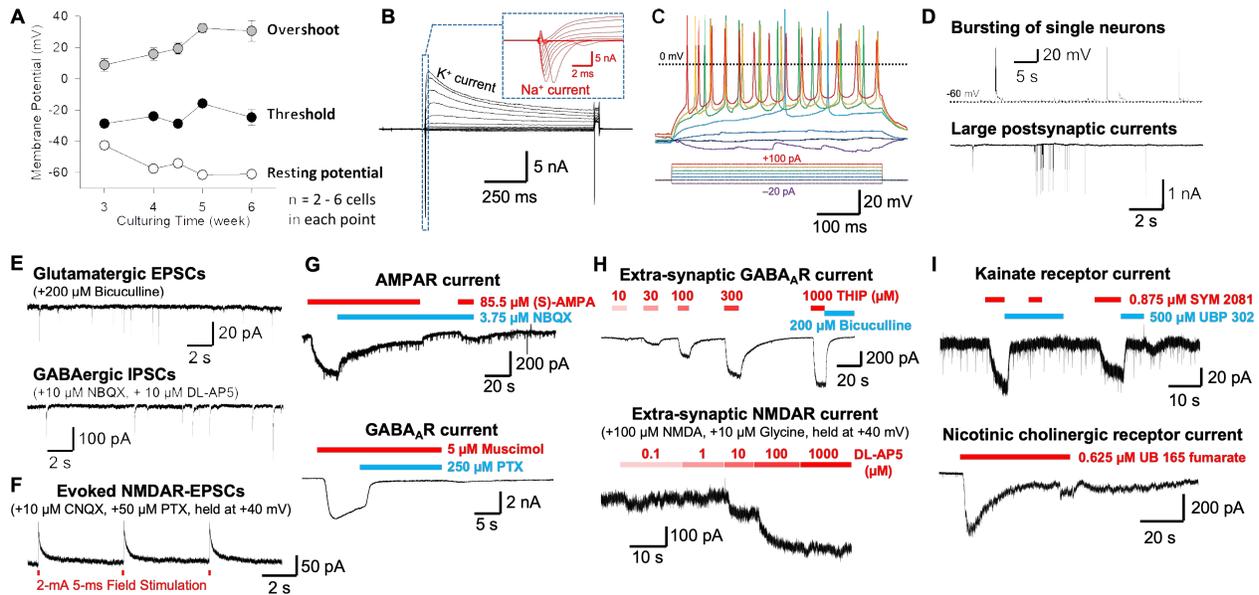
Figure 3. SynFire iNs demonstrate principal neurophysiological properties

(A) SynFire neural cultures rapidly mature, reaching a resting membrane potential ≤ –60 mV within 5 weeks and showing stable excitability (action potential threshold and overshoot). Patch-clamp studies show intrinsic and extrinsic properties in mature SynSire neural cultures, including (B) voltage-dependent K+- and Na+-currents, (C) evoked action potential firings, (D, top) bursting of single neurons, and (D, bottom) large postsynaptic currents indicating advance synaptic competence. (E) Pure SynFire subtype cultures of either (top) only excitatory iNs or (bottom) only inhibitory iNs exclusively show glutamate mediated excitatory post-synaptic currents (EPSCs) or GABA-mediated inhibitory post-synaptic currents (IPSCs), respectively. (F) Showing robust NMDA currents, mature SynFire neural cultures are suited for studying short- and long-term plasticity. (G–I) The function of ionic receptors expressed in SynFire iNs were determined by micro perfusion of their agonists or antagonists, including (G, top) AMPA-, (G, bottom) GABAA-, (H, top) extra-synaptic GABAA-, (H, bottom) extra-synaptic NMDA-, (I, top) kainate-, and (I, bottom) nicotinic cholinergic receptors.
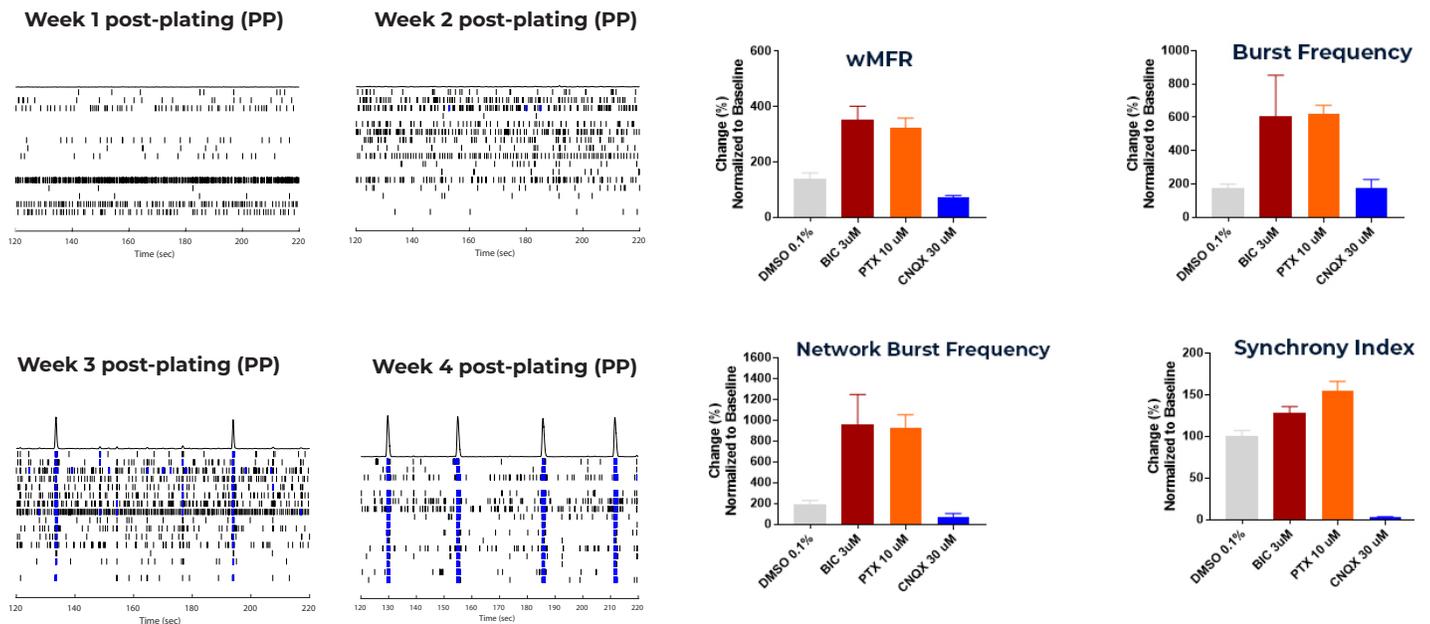
Figure 4. Ontogeny of neural network activity maturation of SynFire co-cultures

These co-cultures contain 70% Glutamatergic, 30% GABAergic neurons and human astrocytes. Representative raster plots from Microelectrode arrays (MEA's) recordings at weeks 1-4. Axion 48 well MEA plates were used to assess activity.

Figure 5. SynFire co-culture responsiveness to GABA and AMPA modulators

Neuronal firing and network activity were assessed in SynFire co-cultures after dosing with the GABA-A blockers Biculline (BIC 3 µM) and Picrotoxin (PTX 10 µM) or the AMPA blocker CNQX (30 µM). Changes in weighted mean firing rate (wMFR), burst frequency, network burst frequency and synchrony index were measured using Axion's MEA plates. GABA blockers have an organizing effect on the network firing. Meanwhile, AMPA blockers cause a break-down in synchronous firing.

Reliable Predictive System for Drug Efficacy and Safety Assessment

NeuCyte's core technology enables the advancement of initial phases of CNS drug discovery programs for lead optimization as well as the investigation of mechanism of action for experimental compounds. NeuCyte Labs's capabilities to make large lots of cryopreserved specific neuronal subtypes is ideal for drug discovery and screening.

SynFire® iN cells represent a versatile in vitro cell system for basic research and disease modeling, including in vitro gain-of-function and loss-of-function genetic studies. The technology can be used to develop in vitro disease models for several neurological disorders with genetic drivers. It also enables the evaluation of human specific neural phenotypes that might not be identifiable in standard animal models.

With the advantages of the SynFire technology, such as rapid maturation and synaptic competence, our human neural in vitro platforms are uniquely suited for assessing relevant complex electrophysiology readouts, which allows better prediction of drug efficacy and potential CNS safety/toxicity than other systems. These cells have been used more and more for compound screening as well as nonclinical safety assessment and chemical neurotoxicity studies.

NeuCyte Labs Supports a Wide Range of Applications

Drug discovery and pre-clinical testing

Custom research line iN generation

Custom in vitro neural disease modeling

Development of neural cell based assays

Phenotypic and targeted drug screening

Neural subtype specific biochemistry

Target identification and validation in biologically relevant tissues

CNS safety/ Neurotoxicity

Cell death and apoptosis assays

Cell stress tests

Neural network physiology assessment (MEA)

**Compound seizurogenic potential testing**

Neurite outgrowth and morphology evaluations

Mechanism of action prediction by gene expression profiling
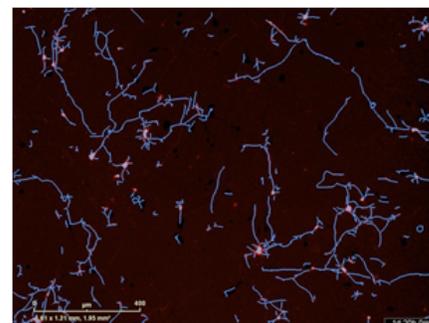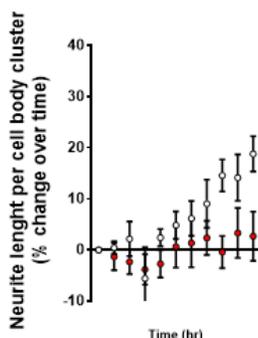
Diverse Platform for Broad Assay Options

NeuCyte's platform is suitable for developing a broad range of functional assays. Neurite outgrowth and seizure liability are two examples below.

Latrunculin A modulation of GABAergic iNs neurite growth
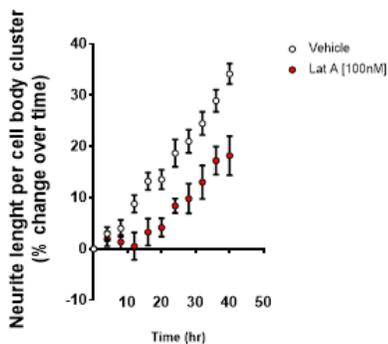
Latrunculin A modulation of Glutamatergic iNs neurite growth

Figure 6. Neurite outgrowth assay using SynFire® iN co-cultures treated with actin filament disruptive toxin
SynFire neural cultures were treated with the actin filament disruptive toxin Latrunculin A (100 nM). Neurite length was assessed and quantified over a period of 44 hours using a live imaging Incucyte system. Representative images of the neurite traces from both excitatory and inhibitory neurons are included.

Compound	0.3µM	1µM	3µM	10µM	30µM	100µM	300µM	1000µM	Human/Animal toxic plasma concentration
4-Aminopyridine									1.1µM
Acetaminophen									331µM
Amoxapine									1.6µM
Amoxicillin									181µM
Bicuculline*									2µM*
Chlorpromazine									1.9µM
Clozapine									3.1µM
Linopirdine*									10µM*
Maprotilene									1.5µM
Picrotoxin*									0.6µM*
Metrazol(PTZ)*									333µM*
Phenytoin									79µM
Pilocarpine									1000µM

* Animal data used

Neurotoxicity scale by MEA measurement: ▮ Low Risk ▮ Medium Risk ▮ High Risk

Figure 7. Seizure liability testing with compounds from the HESI NeuTox MEA seizure prediction initiative using SynFire iN co-cultures
Optimized Synchronized Burst Firing (SBF) signals were measured and used for burst analysis and principal component analysis (PCA).
By comparing with the standard deviation of negative control DMSO, neurotoxicity of chemical compounds can be predicted in a relatively quantitatively scale.**(Data from additional compounds and controls can be found on our website.)**

Please feel free to contact us or visit www.neucytelabs.com/data for additional data.

Proven System Supporting Drug Discovery

SynFire® co-cultures, as an example used in drug discovery, have served to test anti-epileptic drug efficacy and shown better predictive ability than some other iPSC-derived neuronal systems. The progress of NeuCyte's drug discovery programs has further validated this platform.
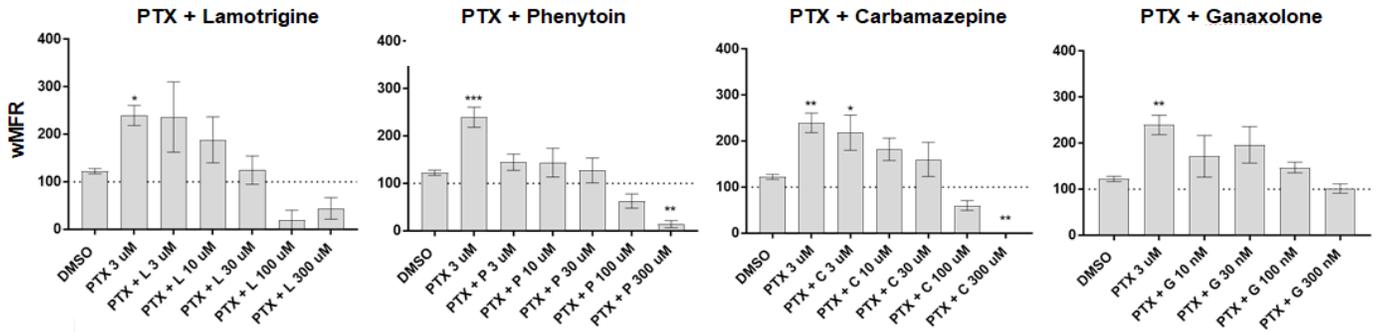
Figure 8. SynFire neural cultures serve to test anti-epileptic drugs (AED) efficacy
NeuCyte's iNs/MEA platform measures quantifiable effects of drugs on neuronal activity. Chemical induced seizure-like activity can be reversed in a dose dependent manner by several AEDs. Assays performed with mixed excitatory/inhibitory iN co-cultures.
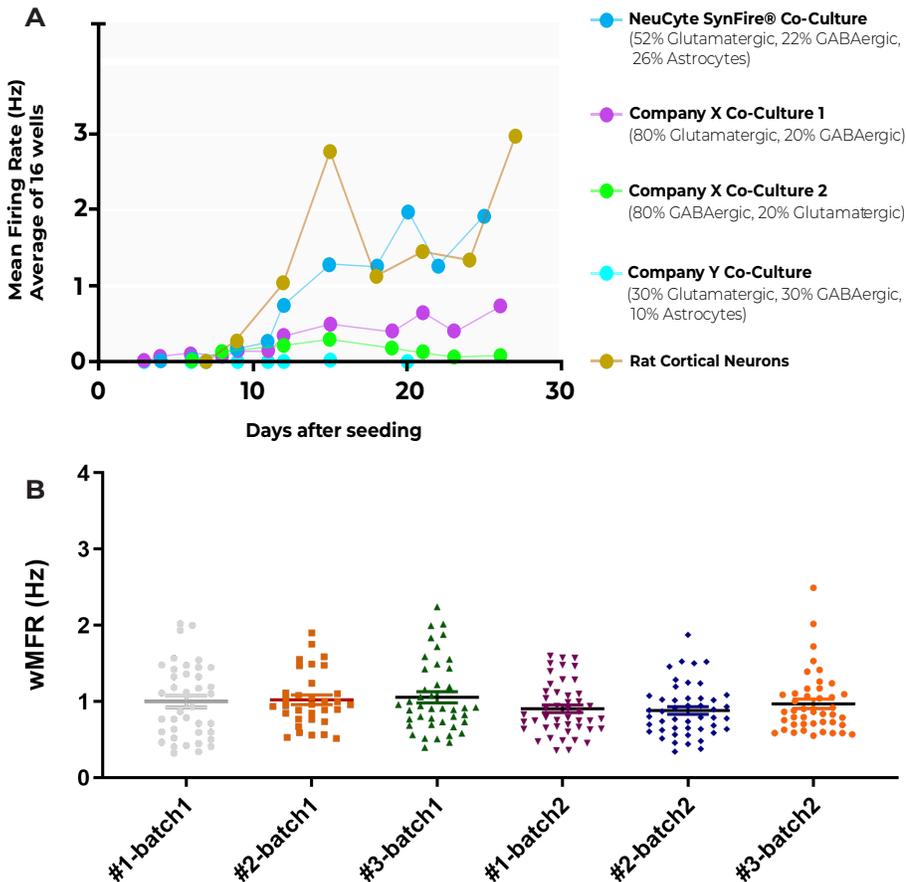
Figure 9. Independent comparison of NeuCyte Labs's SynFire neural cells to other iPSC derived neurons and lot-to-lot comparison
(A) Plot shows the mean firing rate (MFR) of SynFire induced neural co-cultures and other commercially available neurons. MFR was assessed using Axion MEA plates. Axion Maestro Axis software Default setting for spontaneous neuron firing was used (Data provided by customer). (B) Plot shows the weighted mean firing rate (wMFR) of SynFire iNs from multiple batches and different vials from the same batch. Neuronal firing and bursting characteristics show little variability across batches and individuals.

Products, Services & Contact Information

NeuCyte Labs provides highly translatable neural stem cell products and services to enable advancement of CNS drug discovery and development.

Products

SynFire® Line	Pack size
Glutamatergic Excitatory iNs	
GABAergic Inhibitory iNs	**Various sizes and custom packaging available**
Astroglia	
Kits for MEA and other applications	
Media and supplements	

For ordering information, please contact us or go to www.neucytelabs.com/products

Services

Didease Modeling

- Custom iN production
- Research and control lines
- Quality control every step
- Assay development & execution
- Compound screening
- Flexible modular system to fit project and budget needs

In Vitro Neurotoxicity Assesment

- Cell viability and apoptosis assays
- Neural network activity testing (patch clamping and microelectrode arrays)
- Seizure liability testing
- Neurite outgrowth and morphology assessment
- Gene expression analysis

For service descriptions, please contact us or go to www.neucytelabs.com/services

Our goal is to develop applications, assays and protocols to support clients' needs using our technology platform. We always start with the questions you are trying to answer. We have the suitable infrastructure to support drug discovery and nonclinical safety assessment from low to high throughput based on the needs of the individual project. Please contact us with your unique inquiry.

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