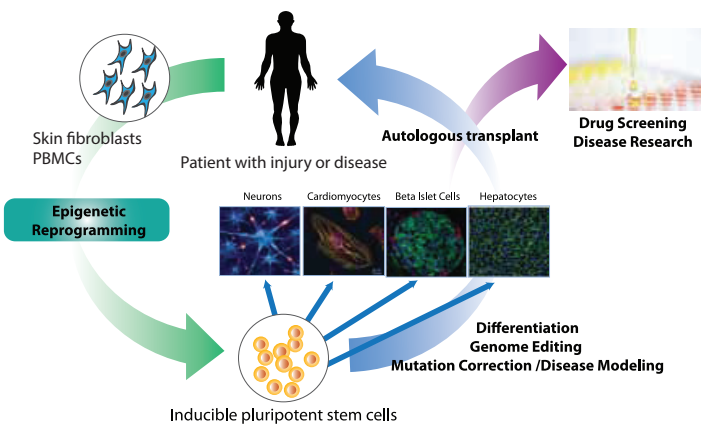
Applied StemCell

ISO 9001 & 13485 Certified

iPSC-based CNS Drug Testing & Neurotoxicity Screening Service

Comprehensive iPSC-based solutions and test battery for drug efficacy and toxicity screening

We Do it All! Applied StemCell (ASC) offers a one-stop-solution for neurological compound screening: generating iPSCs (healthy and diseased patient samples), disease modeling via gene editing, differentiation to cell lineage(s) of choice, cell line model characterization, and a comprehensive cell-based test battery for target discovery, drug efficacy, and neurotoxicity screening.



Improved drug discovery
Reliable safety assessments
Prioritize your drug candidates
Reduce late-stage drug attrition

Key Features:

- Ethically-compatible, physiologically relevant models
- Fast & cost-effective screening
- Regulatory-compliant
- Highly predictive models with reproducible & consistent results
- Stage-specific phenotype screening in a variety of tissues from different sources
- Ready-to-use or custom generated panels of iPSCs & derived cells

Generation of iPSCs lines
Control iPSCs
Engineered iPSCs to model disease
Engineered reporter lines
CRISPR Mutation Correction

Differentiation to Neural Cells
Neural stem cells (NSC)
Neurons (dopaminergic, cortical)
Astrocytes
Oligodendrocytes

Disease Modeling & Drug Screening
Neurotoxicology assays
Neuroprotection screening
CNS drug efficacy testing
Screening for new drug targets

Comprehensive test battery measures multiple morphological and physiological endpoints*

Screening	Types of Assays
Cytotoxicity & Cell Viability Assays	MTT/ MTS cell proliferation assay LDH, Necrosis and Apoptosis assays Luciferase (bioluminescence) expression cAMP level measurement
Mitochondrial Toxicity Testing	Enzyme activity Volume fraction detection
Functional Assays	Calcium influx/ imaging Electrophysiology: Multielectrode array (MEA) analysis and Patch clamp recording
Quantitative Gene Expression	qPCR RNA-seq using NGS (next generation sequencing)
Morphology	Neurite growth assay, Biomarker screening
Custom Assays	iPSC generation; characterization; gene editing; differentiation Custom assay development

** Neurotoxicity screening tests include but are not limited to assays mentioned in table*

Drug Screening Using iPSC-derived Neural Lineage Cells

Application Notes:

1. Using iPSC-differentiated Neurons and Astrocytes in Cell Viability Assays

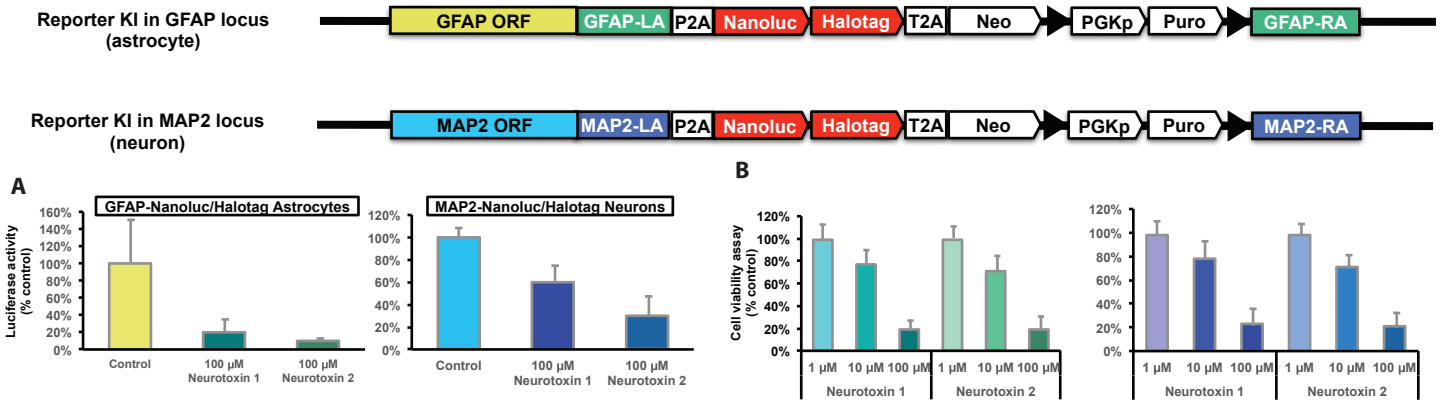


Figure 1A. Luciferase activity (bioluminescence) was used to detect the cell viability of astrocytes and neurons derived from neural reporter iPSC lines (GFAP-Nanoluc/Halotag, ASE-9501; green and MAP2/Nanoluc-Halotag, ASE-9500; blue), respectively, when exposed to 100 µM of neurotoxin 1 and 2. Neurotoxin 1 reduced luciferase activity in astrocytes by ~ 80% and in neurons by ~40%. Neurotoxin 2 reduced luciferase activity in astrocytes by ~90% and in neurons by ~70%. Luciferase activity was measured (as % of control; DMSO-treated cells) to determine the extent of cytotoxicity of the compounds.

Figure 1B. Cell viability of astrocytes and neurons after exposure to neurotoxins. Astrocytes and neurons derived from a control iPSC line (ASE-9109) were exposed to concentrations of 1, 10 and 100 µM concentrations of neurotoxins 1 and 2. Cell viability was evaluated using MTT assay (MTT tetrazolium salt) and cell survival was expressed as % of absorbance of viable cells normalized to control (DMSO-treated cells). The 1 µM concentration of both neurotoxins was not significantly cytotoxic in both cell types while there was mild cytotoxicity observed at the 10µM concentrations of the neurotoxins. The 100 µM concentration of both neurotoxins was significantly cytotoxic and resulted in ~80% reduction in the viability of astrocytes and neurons.

2. Screening drugs for neuroprotection and neurotoxicity using iPSC-differentiated neurons

Table 1. Drugs that were neuroprotective in iPSC and differentiated neuronal cells, and used for human clinical trials

Neurotransmitter/ MAO Inhibitors:	Rasagiline, selegiline, nicotine, topiramate, amantadine, zonisamide, taurine
Antioxidant/ Mitochondrial Stabilizers:	Resveratrol, N-acetyl cysteine, lipoic acid, epigallocatechin gallate, creatine
Anti-inflammatories:	Rolipram, indomethacin, 7-nitroindazole, 3-aminobenzamide, phenanthridone

Table 2. Drug that were not neuroprotective in iPSC-based models but were neuroprotective in conventional cell lines and animal models

Neurotransmitter/ MAO Inhibitors:	Donepezil, caffeine, theophylline, pergolide, apomorphine, riluzole, pramipexole
Antioxidant/ Mitochondrial Stabilizers:	Ascorbic acid, coenzyme Q10, uric acid, folic acid, ropinirole
Anti-inflammatories:	Minocycline, estradiol, clioquinol, plicamycin

Dopaminergic (DA) neurons derived from control iPSC lines were used to evaluate neuroprotection of compounds previously shown to be neuroprotective in rodent and cell line models (Table 1 and 2), when challenged with rotenone or MPP+. Cell viability was measured using the MTT assay. Only 18 out of the compounds (Table 1) were found to be neuroprotective in these iPSC-derived DA neurons, and these same compounds have been used in human Parkinson's disease neuroprotection clinical trials.

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